

CANADIAN JOURNAL OF RESEARCH

VOLUME 13

DECEMBER, 1935

NUMBER 6

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NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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	<i>Annual</i>	<i>Single Copy</i>
A and B	\$ 2.50	\$ 0.50
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The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 13, SEC. C.

DECEMBER, 1935

NUMBER 6

THE EFFECT OF POTASSIUM SUPPLY ON THE COMPOSITION AND QUALITY OF WHEAT. II¹

By A. G. McCalla² AND E. K. WOODFORD³

Abstract

Limiting the supply of potassium to wheat plants resulted in a decreased nitrogen content and a markedly increased calcium and magnesium content of the dry matter. It retarded growth, reduced the total amount of individual nutrients absorbed per plant, and resulted in an increase in the proportion of grain to total yield. It had an adverse effect on the quality of the grain as determined by gluten and baking tests.

Neither sodium nor calcium was effective in replacing potassium, but sodium was the better in this respect.

Introduction

In an earlier paper (9), it was pointed out that the potassium nutrition of the wheat plant markedly influenced the quality of the grain produced, although it apparently affected the nitrogen and mineral content of the kernels but little. The importance of these findings led the writers to repeat the earlier work and, in addition, to study the distribution of nitrogen and minerals in the developing plants.

Representative literature relating to this problem was reviewed in the earlier paper. Since it was written, Hibbard and Grigsby (5) have published the results of a study of pea plants grown in limited potassium and calcium solutions. It was concluded that limiting potassium had little if any effect on the total nitrogen, but that it reduced the ash and increased the calcium content.

Experimental

PRODUCTION OF MATERIAL

The material used in this study was Marquis wheat grown in nutrient solutions during the spring and summer of 1934. Five cultures were produced in tanks measuring 30 × 60 × 8 in. The plants, 512 in each tank, were grown on a seedbed of sawdust spread over burlap in the manner described by Gericke (3). The solutions supplied to the cultures are recorded in Table I, and the chemical composition of the various solutions in Table II.

¹ Manuscript received October 16, 1935.

Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. Published as Paper No. 87 of the Associate Committee on Grain Research of the National Research Council and the Dominion Department of Agriculture.

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TABLE I
SOLUTIONS SUPPLIED TO THE VARIOUS CULTURES

Culture No.	First month	Remainder of growth period	Abbreviated description
1	Complete	Complete	Complete
2	Complete	K-free with Na	Complete : K-free, Na
3	Complete	K-free with Ca	Complete : K-free, Ca
4	Low K with Na	Low K with Na	Low K, Na
5	Low K with Ca	Low K with Ca	Low K, Ca

TABLE II
CHEMICAL COMPOSITION OF SOLUTIONS EXPRESSED AS CC. OF MOLAR SOLUTION PER LITRE

Solution	KNO ₃	Ca(NO ₃) ₂	NaNO ₃	KH ₂ PO ₄	Ca(H ₂ PO ₄) ₂	NaH ₂ PO ₄	MgSO ₄
Complete	5	5	—	1	—	—	2
K-free, Na	—	5	5	—	—	1	2
K-free, Ca	—	7.5	—	—	0.5**	—	2
Low K, Na	—	5	5	0.15*	—	0.85	2
Low K, Ca	—	7.5	—	0.15*	0.42**	—	2

*Added as 0.5 cc. once every 10 days.

**Added as M/16 Ca(H₂PO₄)₂.

The tanks were drained at the end of one month, and refilled with the solutions listed in Table I. At the time of heading the tanks were again drained and refilled. The treatment after heading was therefore quite different from that of the previous experiment in which tap water was used from this stage until maturity.

Ferric tartrate (0.5%) was added to the solutions at the rate of 1 cc. per litre every third day for the first month and then every second day until vegetative growth ceased.

The first seeding was made on April 17, 1934, but when the plants were 44 days old, an accident in the greenhouse killed a large proportion and the series had to be discarded. A second seeding was made on June 2, and the plants grown to maturity.

Sampling was started 20 days after the seeding of the first series. Fifty plants were gathered at random from each tank and brought to the laboratory where they were immediately weighed and analyzed. Similar but smaller samples were collected from the first series at the end of 31 and 44 days, and of the second series after 31 and 54 days. The first heads appeared at the time of the last collection. The plants remaining in each tank were harvested when mature, threshed, and used in composition and quality determinations.

METHODS OF ANALYSIS

With the exception of the determination of potassium, the methods used in this investigation were the same as those employed in earlier work (8, 9). Potassium was precipitated with sodium cobaltic nitrite and the precipitate titrated with *N*/20 potassium permanganate. The details of the procedure are those described by Hibbard and Stout (6).

Results

COMPOSITION OF PLANTS

It was unfortunate that the first series of plants had to be discarded after 44 days, but the second series was grown under similar cultural conditions. The results of the analysis of the two lots of 31-day-old plants are shown in Table III. The actual numerical values for the two series varied somewhat,

TABLE III
COMPOSITION OF 31-DAY-OLD PLANTS FROM THE TWO SERIES

Culture No.	Series No.	T.N., % of D.M.	Ash, % of D.M.	K, % of ash	Ca, % of ash	Mg, % of ash	P ₂ O ₅ , % of ash
1	1	5.43	16.7	41.2	4.15	1.84	16.8
1	2	4.30	13.6	38.0	5.22	1.17	11.2
4	1	4.90	10.0	18.7	8.47	4.56	27.3
4	2	3.90	7.4	17.4	11.13	4.99	21.2
5	1	4.76	9.2	21.6	12.41	5.34	28.0
5	2	3.82	7.2	18.1	15.67	4.76	22.0

but with one exception the order of the cultures was the same for each constituent determined. Since the differences among cultures, rather than the progressive physiological development of the individuals, are stressed in this paper, the variations between the two series of plants are insignificant when compared with the variations among the different cultures.

It seemed preferable therefore to present the results obtained with the developing plants graphically, and to plot the values for the 20-, 31- and 44-day-old plants of the first series with those for the 54-day-old plants of the second series. A break between the points for the 44- and 54-day-old plants has been left in each curve on the graphs, so that the reader will bear in mind that the two series of plants for which these results were obtained were not strictly comparable.

The results dealing with the composition of the developing and mature plants are considered separately because the amount and distribution of any one constituent may be considerably different after vegetative growth ceases than before. This has been demonstrated in earlier work on nitrogen metabolism (8). In deficient cultures, absorption of certain nutrients may con-

tinue as long as there is vegetative growth, but may cease when such growth ceases. On the other hand the same nutrients may be absorbed from a complete solution after vegetative growth is complete. The more important of such effects are noted in the following discussion.

The plants grown in the complete : potassium-free, calcium culture did not develop normally for more than 10 days after the transfer to the potassium-free solution. The roots became dark in color and gradually rotted away. These effects had become quite definite by the time the plants were 54 days old, and probably account for some of the apparently anomalous results presented in the following sections. This culture produced only about 8 gm. of grain, which was insufficient for the various tests carried out on the other samples. Normally the production of grain takes a large proportion of the nitrogen and a considerable proportion of the minerals from the vegetative parts. Since this transfer did not take place, the mature plants were abnormal in composition. All results pertaining to this particular culture are therefore omitted from the tables and discussion dealing with the mature plant.

Nitrogen

Developing plants. The results for total and nitrate-free nitrogen as percentages of the dry matter are presented in Fig. 1. The complete culture plants absorbed a higher proportion of total nitrogen than did those grown in

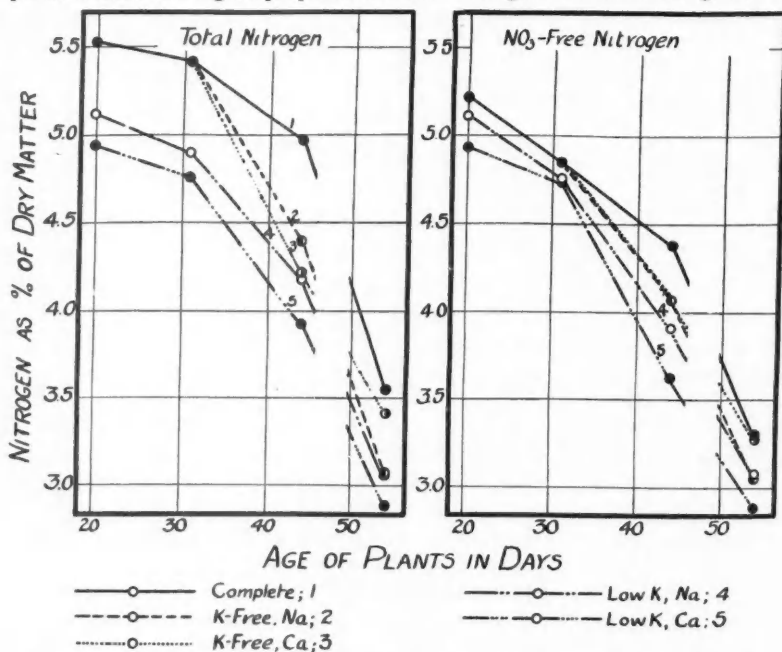


FIG. 1. Total and nitrate-free nitrogen expressed as percentages of the dry matter in relation to age of plants and potassium nutrition.

any of the other solutions. The substitution of sodium for potassium resulted in a greater proportionate uptake than the substitution of calcium. This was true both for the plants grown in the complete solution for one month and then transferred to the potassium-free solutions and for those grown in the low potassium solutions at all stages. The only exception is the nitrogen content of the 54-day-old plants grown in potassium-free, calcium solution and this is believed to have been affected by the relatively poor growth of these plants after their transfer to the potassium-free solution.

The nitrate-free nitrogen was also highest in the complete culture plants, but the difference between the content of these plants and that of any one of the others was considerably less than that found for the total nitrogen. This was of course reflected in the nitrate nitrogen content, this form of nitrogen being present in the complete culture plants in more than twice the quantity found in the plants grown in any other solution. The nitrate content of the various collections varied irregularly, but at the time of the 54-day collection there was no nitrate in the plants from Cultures 2, 4 and 5, while the complete culture plants contained over 7% of their total nitrogen in this form.

The results for organic non-protein nitrogen are shown in Fig. 2. The complete culture plants were definitely lower than the others in this form of nitrogen, and the plants grown in high calcium solutions were higher than the comparable plants grown in solutions in which sodium had replaced potassium. This relation appears to be altered at the time of the fourth collection. Even before this, the development of the plants in the two high calcium solutions had been interrupted. The complete : potassium-free, calcium plants did not mature normally, while the low potassium, calcium plants were small and light.

The higher proportion of non-protein nitrogen has been observed by various workers studying plants grown in potassium deficient solutions. That this proportion is higher in plants grown in low potassium solutions at all stages suggests that the course of metabolism is more disturbed by a continuously low supply of potassium, than by complete absence of this element following a normal supply for one month. The decrease in the non-protein nitrogen content of the 54-day-old plants grown in the high calcium solutions probably

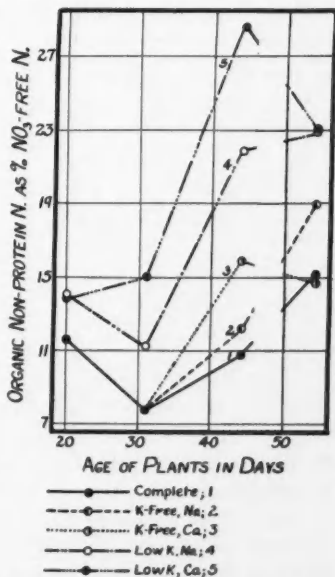


FIG. 2. Organic non-protein nitrogen as influenced by age of plants and potassium nutrition.

does not mean that these plants were becoming more normal in their development, but rather that the lack of potassium was curtailing not only growth, but also absorption, with the result that the non-protein nitrogen present in the plants was being utilized in the production of proteins for new growth faster than it was being formed by the reduction of nitrates. It was found in an earlier study (8) that the removal of nitrogen from the nutrient solution at the time the wheat was heading resulted first in a decrease in the percentage of non-protein nitrogen. This decrease was believed to be directly due to the removal of the non-protein nitrogen already present to form proteins in the developing heads. In the present experiment the same explanation is valid, except that it was potassium supply which was limiting nitrogen absorption.

The deficiency of potassium did not appear to curtail the nitrate reduction in any of the plants, but it did curtail absorption. It also reduced the condensation of the non-protein nitrogen compounds to protein. The last two results are in agreement with those obtained from most other experiments of this type (9).

Mature plants. The total amounts of nitrogen absorbed by the plants remaining in the tanks after samples of the developing plants had been collected are given in Table IV. These figures, which are for the same number

TABLE IV
NITROGEN AND MINERALS ABSORBED BY PLANTS GROWN TO MATURITY

Culture No.	Description	Total N	NO ₃ -free N	Ash	K	Ca	Mg	P ₂ O ₅
<i>As gm. per 100 gm. dry matter</i>								
1	Complete	2.63	2.23	12.56	4.60	1.01	0.12	0.90
2	Complete : K-free, Na	2.14	2.05	7.37	0.85	0.85	0.18	1.04
4	Low K, Na	2.06	1.99	8.18	0.93	0.96	0.15	0.84
5	Low K, Ca	2.33	2.25	8.58	1.42	1.45	0.15	0.89
<i>As total gm. per tank</i>								
1	As above	18.15	15.35	86.70	31.78	6.98	0.80	6.22
2	As above	14.37	13.76	49.46	5.71	5.71	1.24	6.96
4	As above	9.93	9.56	39.35	4.47	4.60	0.70	4.02
5	As above	5.89	5.70	21.70	3.59	3.66	0.37	2.25

in each culture, are referred to as the total absorption of the cultures, although it is recognized that at some stage in the development of the plants there may have been a larger content of some individual element. This is certainly true of potassium, which is known to be excreted during the last weeks of the plant's growth.

The complete culture plants absorbed more total nitrogen per 100 gm. of dry matter than did any of the others, but a larger proportion of the absorbed nitrogen remained in the form of nitrates. The low potassium, calcium culture was as high as the complete in nitrate-free nitrogen, although in the earlier stages it was considerably lower. This change may be attributed to

its restricted production of vegetative material during the later stages of growth. This question is considered more fully in the final section of the paper. The figures for total nitrogen absorption per tank show quite definitely the superiority of the complete culture and the marked inferiority of the culture in which calcium replaced potassium. Apparently the course of absorption was less impaired by the use of the complete solution for one month followed by a complete absence of potassium than by the maintenance of a low potassium supply at all times, although the amount of potassium per 100 gm. of dry weight was actually the lowest in the plants from the complete : potassium-free sodium culture.

Ash and Ash Constituents

Developing plants. The ash results are shown in Fig. 3. The complete culture plants contained much more ash than the low potassium plants, and the difference was maintained throughout the period studied. The percentage of ash in the plants which were transferred from complete to potassium-free solutions at the end of the first month decreased rapidly, and 23 days after the transfer had reached a value almost as low as that of plants which had a limited potassium supply at all stages. The rapid fall in the ash percentage shows the importance of the part played by potassium in the normal absorption by the plant, which is also demonstrated by the marked similarity between the ash and potassium (Fig. 4) curves. The calculation of the correlation between these two constituents gave a coefficient of $+0.96 \pm 0.02$, and although this figure is based on only 16 samples, its significance can hardly be questioned.

The results of mineral analyses expressed as percentages of the dry matter are presented in Fig. 4, and as percentages of the ash in Fig. 5.

The complete culture and low potassium plants maintained a fairly constant potassium level during the time the plants were studied, although the former contained approximately twice as much potassium as the latter. In the plants transferred to a potassium-free solution at the age of one month, the potassium content expressed either as a percentage of the dry matter or as a percentage of the ash, decreased rapidly after the transfer.

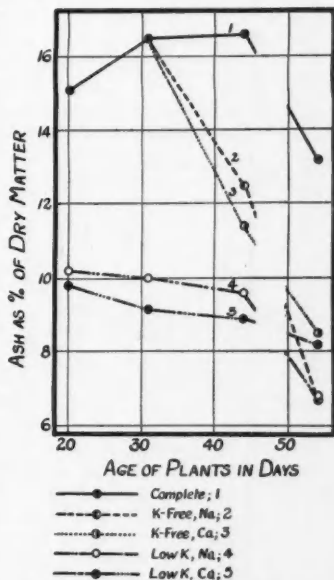


FIG. 3. Ash content of the dry matter in relation to age of plants and potassium nutrition.

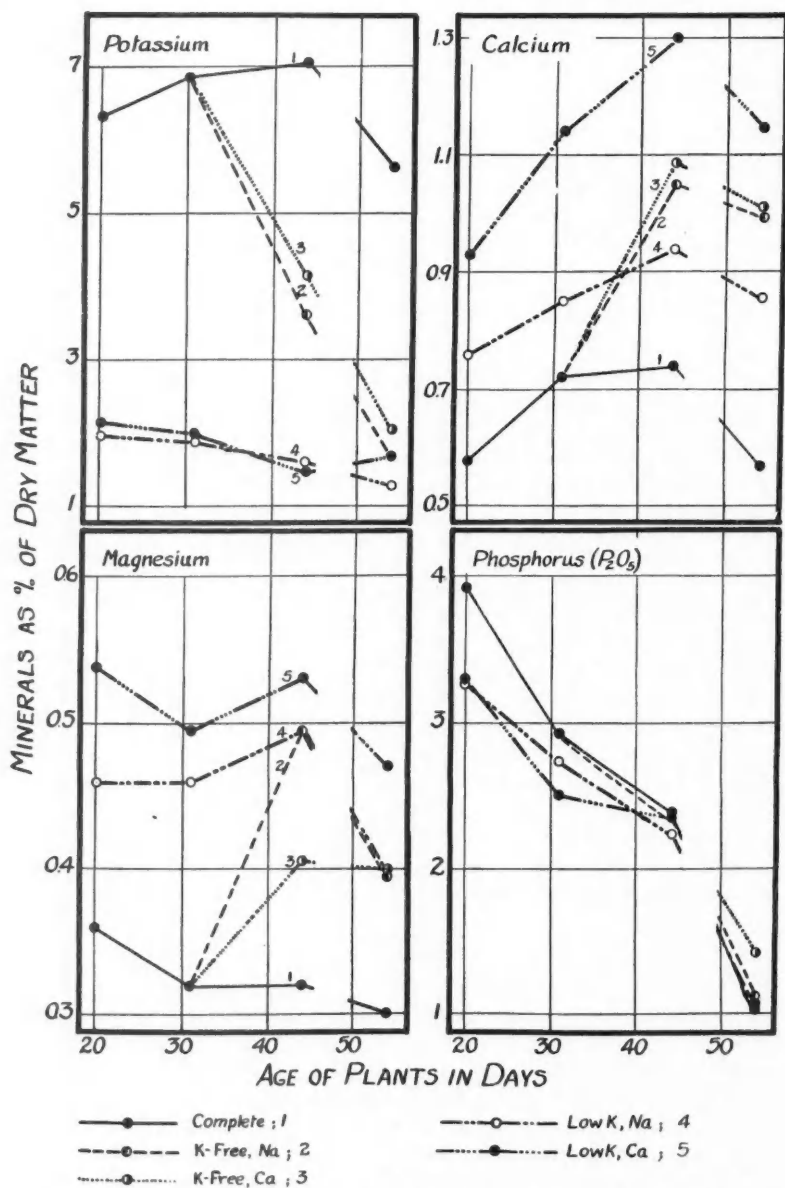


FIG. 4. The principal minerals expressed as percentages of the dry matter in relation to age of plants and potassium nutrition.

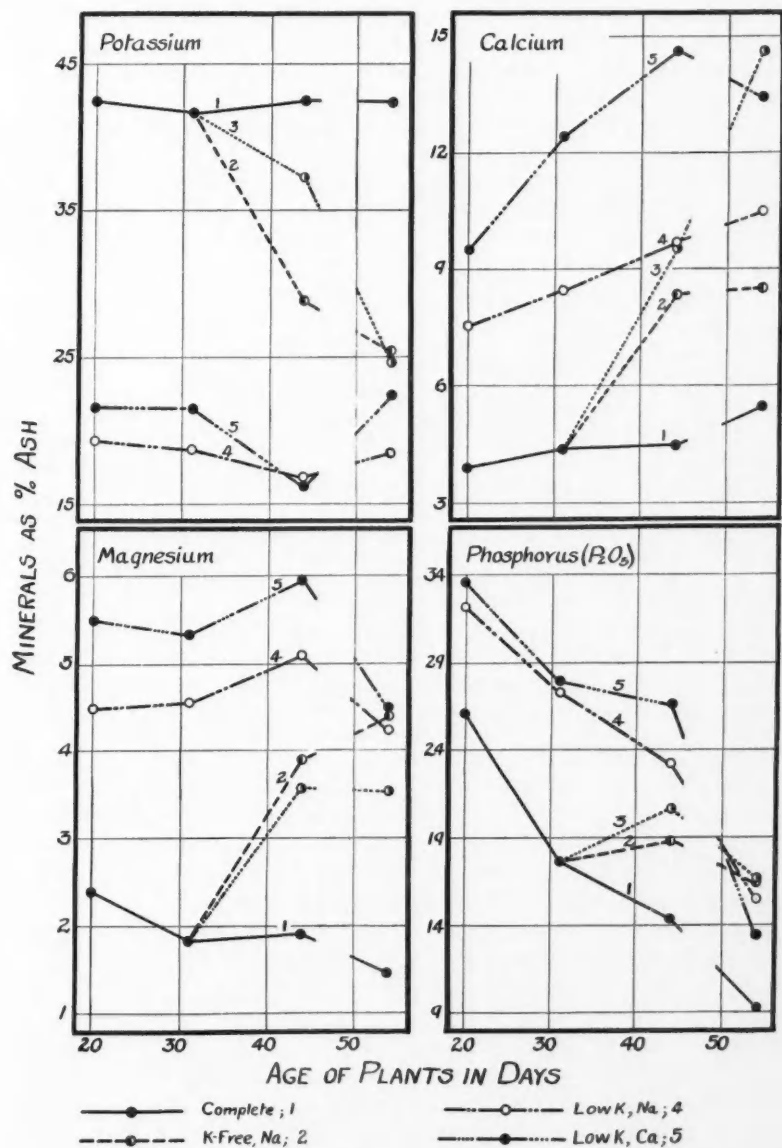


FIG. 5. The principal minerals expressed as percentages of the ash in relation to age of plants and potassium nutrition.

The effect of limiting potassium supply was reflected in the calcium and magnesium content of the dry matter and ash. The complete culture plants were decidedly the lowest in their content of both these cations, while the plants grown in the low potassium, sodium solution absorbed considerably less calcium and slightly less magnesium than did those grown in the low potassium, calcium solution. The plants grown in Cultures 2 and 3 showed a rapid rise in the percentage of calcium and magnesium during the first two weeks after the transfer from the complete solution. Sodium seemed to be less effective in replacing potassium in these plants than in those supplied with low potassium solution at all stages.

The absorption of phosphorus was less affected by the modification of potassium supply than was the absorption of other mineral elements. On the basis of percentage dry matter there were but small differences in phosphorus content of the various plants, although those grown in the complete culture were higher during the early stages. On the basis of percentage ash, however, the phosphorus content of the complete culture plants was definitely lower than that of the plants grown in low potassium solutions. When the plants were transferred from the complete to potassium-free solutions, the phosphorus content of the ash increased, and at the time of the final collection it was higher in these plants than in any others. If the phosphorus results are expressed as percentages of the potassium-free ash, the differences among the cultures are greatly reduced, but are still marked.

The restriction of potassium therefore resulted in a definitely increased proportional absorption of calcium and magnesium both in relation to dry matter and total ash, and in an increased absorption of phosphorus in proportion to total ash but not in proportion to dry matter.

Mature plants. The amounts of the various ash constituents present in the mature plants are given in Table IV. The ash values were all higher than in the earlier experiment (9). The difference between the ash uptake of the complete and that of any one other culture was more than the difference in the uptake of potassium, indicating that the general absorption of mineral constituents was impaired by potassium restriction.

In all but the complete culture plants, potassium absorption was largely determined by potassium supply. Although, as already stated, the plants grown in the complete : potassium-free, sodium culture contained the least potassium per 100 gm. of dry matter, this culture absorbed more during the first month of development in the complete solution than did the low potassium cultures throughout their growth.

More calcium per 100 gm. of dry matter was absorbed by the low potassium, calcium culture than by any of the others, but the other restricted potassium cultures were lower than the complete. This was quite a different situation than with the developing plants where the percentage of calcium was lowest for the complete culture. Obviously, these plants absorbed much more

calcium after heading began than did the plants in the other cultures. This demonstrates the effect which the supply of one element may have on the absorption of another. Despite the differences in relative absorption, the complete culture plants absorbed more, and the low potassium, calcium plants less, calcium than did the other two cultures.

As in the previous experiment, the differences in the magnesium content were smaller than those for potassium and calcium, but the complete culture appeared to be significantly lower and the complete : potassium-free, sodium culture higher than the others.

The phosphorus results were similar to those for magnesium in that the complete : potassium-free, sodium culture was the highest both in weight per 100 gm. of dry matter and in total absorption. Complete absence of potassium apparently induced an increase uptake of phosphorus in proportion to the total production of dry matter. In the earlier experiment (9) high phosphorus absorption accompanied high calcium uptake, but in the present work, the phosphorus content of the culture in which calcium replaced potassium was no higher than that of the check.

The magnesium and phosphorus results for developing and mature plants differed as did the nitrogen and calcium results. Undoubtedly they were also affected by the absorption of these elements after vegetative growth had practically ceased or heading had started.

Although there are minor differences between these and previous results, the general conclusion that restricted potassium supply decreases total absorption is substantiated. The conclusion that the composition of the culture solution does not wholly determine the composition of the plants is also borne out. The selective absorption noted in the previous experiment was again manifested, for although the ratio of potassium to calcium in the low potassium solutions was as low as 1:50, the ratio of absorbed potassium and calcium was approximately 1:1.

The general development of the plants in the present experiment was not as good as in the previous year. The late start and consequent subjection to hot summer conditions during the early stages of development probably caused the lower dry weight per plant, but is believed to have had no marked influence on the differential behavior of the cultures.

It should be noted that the restriction of potassium supply in this experiment not only affected chemical composition but definitely retarded growth (Table V). The substitution of sodium for potassium was less deleterious than the substitution of calcium, whether the plants were grown in the low potassium solutions at all times or transferred to potassium-free solutions after having an unrestricted supply for one month. Sodium, however, did not compensate for the low potassium either in maintaining growth or in absorption. These results are in good agreement with other work on absorption and the effect of the replacement of one ion with another (4).

Yield and Grade

The results for yield per tank, grade and weight of grain are presented in Table V. The sample (Culture 4) with the highest grade also had the highest weight per bushel and weight per thousand kernels. The grain from this

TABLE V
YIELD, GRADE AND WEIGHT OF MATURE MATERIAL

Culture No.	Description	Yield, gm. per tank			Grade of grain	Weight per bushel, lb.	Weight per 1000 kernels, gm.
		Straw	Grain	Total			
1	Complete	539	152	691	1 Northern	61.5	27.24
2	Complete : K-free, Na	467	204	671	1 Northern	61.5	29.61
4	Low K, Na	308	173	481	1 Hard	63.0	33.58
5	Low K, Ca	170	83	253	1 Northern	61.5	25.53

culture in the previous experiment had very small kernels, with high grade and weight per bushel. The results afford no explanation as to why the grain from this low potassium, sodium culture should have differed so markedly in the two years.

The yield results are particularly striking as they show that the total dry matter produced by the complete culture was the highest, but that the weight of grain from this culture was less than from the two in which restricted potassium was replaced by sodium. This lower proportion of grain to straw in complete culture plants has been noted before (8, 9), as has the fact that it is accompanied by a shrivelling of the kernels. The uptake of nitrogen after heading was considerably greater in this than in the other cultures, and the high nitrogen absorption, taking place after the dry weight has reached a maximum, apparently results in a shortage of soluble carbohydrates for translocation to the developing grain (8). This results in a high nitrogen content of the kernels, but a reduced yield of grain. This effect has been obtained in three distinct experiments, and seems to be fully established.

The low yield from the low potassium, calcium culture indicates that substitution of calcium for potassium was more deleterious than a similar substitution of sodium. The fact that the complete:potassium-free, calcium culture failed to develop normally supports this conclusion. The solutions in which calcium was substituted for potassium contained no univalent cation, and it is possible that the beneficial effect of sodium as compared with calcium may be an effect of maintaining a physiologically balanced solution.

Composition of Grain

The results of the analyses of the grain are presented in Table VI.

Nitrogen as a percentage of dry matter of the grain was closely related to the amount of this element absorbed per 100 gm. of dry matter. This fairly constant proportional translocation of nitrogen to the kernels has been discussed before (9), and the present results support the conclusion that regardless of the amount of total nitrogen absorbed, about 75% of it will be found

TABLE VI
COMPOSITION OF GRAIN AS PERCENTAGE OF DRY MATTER

Culture No.	Description	N	Ash	K	Ca	Mg	P ₂ O ₅
1	Complete	3.34	2.75	0.74	0.10	0.21	1.29
2	Complete : K-free, Na	2.83	2.40	0.54	0.08	0.20	1.19
4	Low K, Na	2.71	2.32	0.52	0.08	0.21	1.06
5	Low K, Ca	3.24	2.41	0.64	0.11	0.21	1.38

in mature kernels, and the percentage nitrogen content of the kernels will be determined by the relation between yield and nitrogen absorption. Thus the nitrogen content of the grain from the low potassium, calcium culture was almost as high as that of the grain from the complete culture, although the total absorption and yield were much lower.

The ash content of the grain also showed a rough agreement with the absorption by the whole plant, but this relation was less marked than with nitrogen. There was a selective translocation which operated to keep the mineral composition of the grain more nearly uniform than the composition of the whole plant. The potassium content of the kernels was roughly parallel to the nitrogen content, but the calcium and magnesium results showed little variation. The phosphorus content was also roughly parallel to the nitrogen content, a fact in agreement with previous work (9).

Quality of Grain

In the previous experiments, quality was estimated by studying the distribution of nitrogen in the grain, and by examining the physical properties of the gluten. In the present experiment these methods were supplemented by a baking test carried out on finely ground, whole-wheat meal.

The results for nitrogen distribution are presented in Table VII. The outstanding feature of these results is that the grain from the low potassium, calcium culture contained markedly less gluten nitrogen in proportion to total nitrogen than did the grain from the other cultures. The value 1.06% of the dry matter as non-gluten nitrogen is higher than has been previously obtained by the writers with widely varying material, and demonstrates the failure of the lower nitrogen compounds to condense to form gluten.

TABLE VII
DISTRIBUTION OF NITROGEN IN GRAIN

Culture No.	Description	Total nitrogen, % D.M.	Nitrogen in gluten		Nitrogen washed from meal	
			% T.N.	% D.M.	% T.N.	% D.M.
1	Complete	3.34	79.0	2.64	21.0	0.70
2	Complete : K-free, Na	2.83	73.6	2.08	26.4	0.75
4	Low K, Na	2.71	78.3	2.12	21.7	0.59
5	Low K, Ca	3.24	67.4	2.18	32.6	1.06

The physical characteristics of the gluten are recorded in Table VIII. The highest yield of wet gluten was obtained from the grain grown in the complete solution. Although the results are not very definite, the lower dry matter

TABLE VIII
PHYSICAL CHARACTERISTICS OF GLUTEN

Culture No.	Description	Weight of wet gluten, gm. per 10 gm.	Dry matter in gluten, %	Nature of wet gluten			
				Elasticity	Extensibility	Spring	Color
1	Complete	6.75	33.5	Very good	Good	Very good	Very good
2	Complete : K-free, Na	5.80	35.0	Fair	Fair	Fair	Fair
4	Low K, Na	5.90	34.3	Good	Good	Good	Poor
5	Low K, Ca	6.14	34.4	Fair	Poor	Fair	Fair

content of this gluten suggests that it is of better quality than the others. More extensive studies have shown that better quality gluten usually retains more water than does coarser, more open gluten. The gluten from the grain grown in the low potassium, calcium culture had the poorest quality of the four studied.

The wheat-meal baking test was carried out by baking a mixture of 75% whole wheat meal with 25% of a weak pastry flour, using 50-gm. samples and the malt-phosphate-bromate formula described by Aitken and Geddes (1). The details of this method will be published later when more extensive tests have been carried out. Preliminary work has shown that this procedure gives results in good agreement with those obtained when flour milled from the same sample is baked. The method has been used with several series of named varieties of hard wheat, and has consistently placed the varieties in their accepted order for quality. Calculation of the deviation between duplicates has shown that a difference of 14 cc. between the volumes of two loaves is significant.

The baking results obtained from the present series show that the grain from the complete culture plants yielded a loaf at least 25 cc. larger than that baked from the grain produced by any of the other cultures. There were no significant differences among the other results, but there seems to be no doubt that restricting potassium supply decreases the strength of the wheat as measured by the volume of the bread.

TABLE IX
BAKING TEST

Culture No.	Description	Loaf volume, cc.	External form	Crumb texture
1	Complete	309	Good	Good
2	Complete : K-free, Na	276	Flat	Fair
4	Low K, Na	282	Flat	Fair
5	Low K, Ca	284	Very good	Good

The weakness in external form and texture of the samples from the cultures in which sodium was used was expected from the results of the study of the gluten, but the form and texture of the bread baked from the other restricted potassium sample were better than anticipated.

The quality studies in general show that the grain from plants grown in the complete solution was superior to the other samples, but do not permit definite differentiation among the latter. Had the growth of the plants produced in the low potassium, calcium culture not been impaired to such an extent that the nitrogen content of the grain was higher than that of the other limited potassium cultures, there is reason to believe that the quality would have been lower than in the cultures where sodium was used to replace potassium. This was the conclusion reached as a result of the first year's study.

Discussion

The results of this experiment are in agreement with the previous year's work. Limiting potassium supply retarded growth, reduced the uptake of inorganic elements and greatly altered the chemical composition of the whole plant. It also had a harmful effect on quality, although the composition of the grain was not altered as much as the composition of the rest of the plant. Neither sodium nor calcium was effective in replacing potassium, but sodium was the better in this respect.

In interpreting results of an analytical study such as this, care must be taken to consider both percentages and total weights. The importance of this point has been discussed by Bartholomew, Watts and Janssen (2), who point out some of the erroneous conclusions reached as a result of the study of percentage data only. Hibbard and Grigsby (5) considered only percentage data in interpreting their results on nitrate accumulation in pea plants grown in potassium-deficient solutions. These plants contained more nitrate than did similar plants grown in a complete solution, although the percentage of total nitrogen was approximately the same in both cases. Knowing that there was an abundant supply of carbohydrates in both deficient and normal plants, the authors concluded that the higher nitrate content was due to a greater nitrate absorption. If the total weight of nitrogen absorbed is calculated, however, it is found that in every case the complete culture plants absorbed more nitrogen than the deficient plants. Since all the nitrogen was supplied as nitrate, the higher nitrate concentration could not be due to a greater absorption, and was in all probability caused by a failure of the nitrate-reducing mechanism of the deficient plants.

The effect of potassium nutrition on the course of nitrogen metabolism has been studied by many workers, but the results are not in good agreement. It has been variously reported that the percentage nitrogen content of potassium-deficient plants is lower (4), higher (7) and the same (5) as that of complete culture plants. This percentage of nitrogen is obviously determined by the relation between total growth and total nitrogen absorption. In the present experiment, Culture 5 was so retarded in growth, by restricting the

potassium supply, that the percentage of total nitrogen was almost as high as that of the plants grown in the complete culture, although the total absorption was less than one-third. This effect was more accentuated in Culture 3, which was discarded because of the failure to produce seed, and the percentage of total nitrogen was actually higher than that of the check, although the total absorption was lower than that of any of the other cultures. In Cultures 2 and 4, where the limitation of potassium did not retard growth as much, the percentage of nitrogen was considerably lower than that of the checks. Thus the disagreement among the results of other workers may possibly be explained by the relative effects of potassium deficiency on growth and nitrogen absorption.

Just as the effect of nutrition on the metabolism of the plant cannot be determined by the study of percentage data alone, neither can the effect of nutrition on the quality of the grain be studied by analyzing only the grain. This point was discussed in the earlier paper, and the results of the present experiment are in agreement. The effects of nutritional disorders are often manifested to a much greater extent in the composition of the straw than of the grain, and if reliable conclusions are to be drawn from this type of study the composition of the grain in relation to the rest of the plant must be considered.

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STUDIES ON THE NATURE OF DISEASE RESISTANCE IN CEREALS

III. THE ORGANIC NITROGEN CONTENT OF MATURE AND IMMATURE TISSUES OF THE WHEAT PLANT IN RELATION TO STEM-RUST RESISTANCE¹

BY T. JOHNSON² AND O. JOHNSON³

Abstract

Analyses were made to determine the organic nitrogen content of immature and mature tissues of six wheat varieties at a stage of growth about a week prior to the emergence of the flag leaf. The immature tissues comprised the leaves folded within the uppermost sheaths, while the mature tissues were represented by the fully grown leaves of the upper half of the plants. In all the varieties the nitrogen content of the mature tissues was greater than that of the immature ones. The greater susceptibility of the younger tissues can not, therefore, be attributed to a higher organic nitrogen content.

Introduction

The young, rapidly developing tissues protected by the sheaths of the growing wheat plant have been shown by Newton and Brown (3) to be more susceptible to stem rust (*Puccinia graminis tritici* Erikss. and Henn.) than exposed, fully grown plant parts. Analyses for sugar content (2) showed that the young tissues (leaves and immature stem parts still enclosed by the sheaths) were considerably richer in sugars than the older plant parts, that is, than the fully grown, but still photosynthetically active, leaves and sheaths.

It has been recognized for some time that nitrogenous fertilization is capable of increasing rust development on cereal plants. Recently, Gassner and Franke (1) have attributed this phenomenon to an actual increase in the nitrogen content of the plants. In moderately resistant cereal plants they found that an increase in the nitrogen content led to an increased susceptibility to rust. Accordingly they maintain that an excess supply of nitrogen increases the nitrogen content, and consequently the protein content, of the host plants and thereby makes available to the rust organism a greater amount of the protein elements requisite for its development.

The present authors have analyzed mature and immature tissues of the wheat plant to ascertain whether or not the immature tissues, which are more susceptible to stem rust, are richer in organic nitrogen than the more resistant, mature tissues. If such were shown to be the case there would be reason to suppose that the higher nitrogen content was a factor contributing to the greater susceptibility of the immature tissues.

Six wheat varieties were selected for analysis. On the basis of rust reaction these varieties may be divided into three groups. (i) Hope, R.L. No. 716,

¹ Manuscript received October 26, 1935.

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and Pentad possess the so-called "mature-plant resistance". In the seedling stage they are moderately resistant or even susceptible to many physiologic forms but develop a high degree of resistance as they approach maturity. (ii) Iumillo in all stages of growth shows high resistance to stem rust. (iii) Marquis and Little Club may be classed as susceptible, the latter variety being susceptible to the great majority of the known physiologic forms of wheat stem rust. In all these varieties, with the apparent exception of Iumillo, the rapidly growing tissues protected by the sheaths are susceptible to physiologic forms to which their more mature plant parts are resistant. In Iumillo even the younger tissues appear to be highly resistant.

Methods

The six wheat varieties were grown in the field in rod rows. Material was collected for analysis at least one week prior to the emergence of the flag leaf—at a stage when most of the culms, including tillers, bore four or five leaves. All collections were made between 1:00 and 2:00 p.m. The young tissues comprised the leaves folded within the uppermost sheaths of the plants while the mature tissues were represented by leaves fully grown at the time of collection. These plant parts correspond to those previously analyzed for sugar content (2). The method of collecting the two types of tissue was the same as that employed in the earlier work, the leaves being stripped off and dropped into a covered glass jar lined with moist blotting paper, while the young tissues were separated out rapidly and dropped into a similarly prepared glass receptacle. Two 15-gm. samples of young and of mature tissue of each wheat variety were then dried rapidly in an "air-blast" oven at 85° C., after which the dried material was used for analysis. An analysis of the duplicate samples of both types of tissue was carried out according to the Kjeldahl method for organic and ammonia nitrogen as given in the A.O.A.C. p. 21, 1930. The results are given in Table I.

Experimental Results

Table I shows that in all the varieties tested the nitrogen content of the older tissues is greater than that of the younger. The greater susceptibility of the latter tissues to rust can not therefore be attributed to a higher organic nitrogen content. It follows that the above-mentioned conclusions of Gassner and Franke are not applicable in the case of the tissues under discussion unless it is assumed that the organic nitrogen of the younger tissues is present in a form more utilizable to the fungus than that of the older plant parts. There is, of course, a possibility that the greater nitrogen content of the older tissues fails to induce susceptibility to rust through interference by some unknown factor such as the development, in the more mature tissues, of a substance inhibitory to the growth of the rust organism. It does not seem probable, at any rate, that the resistance to rust developed by the more mature tissues of the wheat plant can be explained on any purely nutritional basis.

TABLE I
PERCENTAGE OF ORGANIC NITROGEN IN YOUNG AND OLDER TISSUES OF WHEAT VARIETIES

Wheat variety	N as % dry weight		N as % green weight	
	Immature tissues	Mature tissues	Immature tissues	Mature tissues
Hope	2.60	3.85	0.403	0.770
	2.58	3.85	0.400	0.770
R. L. No. 716	2.82	3.64	0.400	0.870
	2.84	3.58	0.409	0.824
Pentad	2.44	3.01	0.349	0.542
	2.35	2.96	0.336	0.553
Iumillo	2.64	3.73	0.375	0.627
	2.56	3.55	0.369	0.649
Marquis	2.70	3.60	0.400	0.742
	2.68	3.82	0.402	0.796
Little Club	2.60	4.03	0.369	0.649
	2.46	3.71	0.335	0.671

Acknowledgment

The authors are indebted to the Department of Chemistry of the University of Manitoba for the use of its Kjeldahl apparatus.

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MICROBIOLOGICAL STUDIES OF APPALACHIAN PODSOL SOILS

II. SEASONAL CHANGES IN MICROBIAL ACTIVITY¹

By P. H. H. GRAY² AND H. J. ATKINSON³

Abstract

Seasonal fluctuations in numbers of micro-organisms in soil, estimated by the plate method for bacteria and actinomyces and by the evolution of carbon dioxide, have been studied with samples of cultivated podsol soils, drawn from experimental fields under various treatments at three farms in the Eastern Townships region of Quebec, at intervals of six weeks during the cropping season in two years. The nature of the fluctuations in numbers of micro-organisms was similar in the three soils, which were situated many miles apart. In 1931 numbers were lower in summer than in spring and autumn; in 1932 numbers fell towards the end of the season. The evolution of carbon dioxide fluctuated to a comparatively less extent than the bacterial numbers, but there was general agreement in the direction of the changes in the two years. Biological activities in any one soil were at significantly different levels in the two years. There was an agreement between numbers of micro-organisms and the amount of carbon dioxide, in that changes in both of these factors were in the same direction in the second year of sampling; there was also evidence of agreement between intra-annual changes in numbers and carbon dioxide, especially in the alkali-treated plots. Fluctuations in numbers and carbon dioxide in samples from plots in different parts of the same field or farm were in conformity, and were superimposed on the effects of chemical treatments that caused significant increases in microbial activity.

Introduction

The fluctuations of bacterial numbers in field soil, at short intervals of time, have been found to be of sufficient amplitude to make isolated determinations of numbers valueless except for comparisons of such treatments as can be shown to disturb the normal equilibrium of the soil population (19). It may, nevertheless, be true that the difference in general level of numbers between two seasons of the year may be sufficiently great to be accepted as significant and as being due to factors inherent in a changing microflora. The acceptance of these changes as due to causes other than those of random sampling can be justified only after a study of a sufficiently large number of samples.

The changes of bacterial numbers found by Cutler, Crump and Sandon to occur daily in field soil supply the most reliable evidence of intra-annual and inter-annual changes, to which, however, no adequate causes could be ascribed (2). It is clear, from their graphs of fortnightly averages, that the bacterial numbers at midsummer, 1921, were about double those found at midsummer, 1920. Similar inter-annual differences in bacterial numbers in field soils were found by Crump in samples taken at weekly intervals during 1916 and

¹ Original manuscript received August 22, 1935.

Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 68. This paper is the second in a series dealing with part of the work of the Soil Fertility Committee of Macdonald College, the names of the members of which were given in the previous paper (Macdonald College Journal Series No. 63, in the Canadian Journal of Research).

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1917 (1); in this case, however, it was suggested that soil moisture could be accepted as one of the causes contributing to the intra-annual (weekly) changes (6). The work of Erdman, in Iowa, also showed that numbers of bacteria may be at considerably different levels in the same season of two years (4).

Newton found that bacterial numbers in Alberta soils fluctuated extensively, and confirmed the general view that numbers are lower in summer than in spring and autumn; the results also suggest that the average numbers in two years were distinctly different (10). The results of Gräf, though not based on an extensive series of counts, are in conformity, in that numbers fell during the summer, and are of added interest in that the numbers near to and away from plant roots, though at considerably different levels, were affected similarly by the season (5). This statement applies to the normally mixed population of colonies appearing on a moderately selective medium. On the other hand, she reported that the numbers of a specific group, *Radiobacter*, increased rapidly during July and August.

The seasonal fluctuations of numbers and activity in specific groups of micro-organisms have been demonstrated conclusively by the use of partially selective mediums; less selective mediums tend to mask the fluctuations that may exist in smaller groups (18). In this connection the results of Vandecaveye and Anderson are of much interest; they found that *Azotobacter* was present in soil in varying numbers in spring, vanished in June, July and August, and returned to show further seasonal fluctuations until November in one year (20). They suggested that although this organism can be shown to fluctuate extensively in regard to numbers, the numbers counted may not be contributing anything to the soil's store of fixed nitrogen, at least not until their numbers reach a much higher level than the maximum reported to have been reached that year. This is a point of importance in any discussion on the probable significance of changes in numbers in specific groups of micro-organisms.

The numbers of bacteria in a soil are to some extent dependent upon the presence of higher plants, and the distance from the latter at which a sample is taken, as was shown by Gräf in the work referred to above. The work of others has been reviewed by Starkey, who gives further evidence of the effects of living plant roots on the numbers of micro-organisms and on the evolution of carbon dioxide during one growing season in which fluctuations occurred in both fallow and cropped soil. The best evidence of seasonal fluctuations in bacteria and other micro-organisms rests upon results of sampling fallow soil (2, 19), or soil now or recently under grass (3, 16). In a previous paper the present authors have shown that when the method of sampling adopted in this work was used, the numbers of bacteria in cropped plots receiving no treatment were not different from those in fallow plots (7). The results submitted below would seem to suggest that seasonal fluctuations may be superimposed on changes due to treatments which are even more efficient in their effects upon the microbial equilibrium than is the growth of plants.

The same considerations apply to fluctuations in the evolution of carbon dioxide by soil micro-organisms, determined by methods that take into account or eliminate the possible influence of growing plants. A large proportion of the carbon dioxide in soil air is due to micro-organisms decomposing the organic residues, for comparisons of fallow and cropped soil seem to indicate little or no significant differences (7, 17). Seasonal changes that can most reasonably be ascribed to micro-organisms have been reported to occur intra-annually by Jodidi and Wells (8), Russell and Appleyard (12, 13), Potter and Snyder (11), and by Smith, Brown and Millar (15). The results of Russell and Appleyard are of interest in the present work, because these workers determined the respiratory intensity of soil in manured and unmanured field plots for two years, and in several instances determined both bacterial numbers and amount of carbon dioxide on the same day, though only in the latter half of the two summers, *viz.*, July 11 to Sept. 22, 1913; July 7 to Sept. 21, 1914. The amounts of carbon dioxide decreased considerably between the dates mentioned, the results for the two years being parallel, and there was slightly more activity in the second year. The results of Potter and Snyder show the need for continuous rather than intermittent sampling; their results from thirteen plots, when averaged, show clearly the differences that might be obtained by taking samples at intervals shorter than six weeks.

The present work was planned to determine the extent of microbial activity in soils more widely separated than it has been usual to select, to ascertain if such intra-annual and inter-annual fluctuations would prove to be local or to occur in a larger area under similar climatic conditions.

Experimental

The soils selected were three that fall into the group of heavily leached (podsol) soils of the Appalachian uplands of Quebec, certain characteristics of which have been described (9, 14); further details relative to the actual soils used are submitted below. The figures in Table I represent the average

TABLE I
SOME CHARACTERISTICS OF THE SOILS UNDER INVESTIGATION
(Figures, except those for lime requirement and pH, represent percentages on a basis of moisture-free soil.)

	Brome County	Compton County	Sherbrooke County
Lime requirement (lb. CaO per acre)	4117	5095	6183
pH	5.46	5.41	5.37
Moisture	1.99	2.61	2.77
Loss on ignition	7.81	9.08	9.74
Carbon (organic)	3.37	4.09	4.44
Total nitrogen	.247	.284	.282
Phosphorus (P_2O_5)	.294	.162	.141
Silica (SiO_2)	72.17	72.36	73.43
Calcium (CaO)	.97	.54	.62
Magnesium (MgO)	.77	.57	.68

values of composite samples of 60 cores each. These were taken to a depth of 6 inches, from the normally ploughed half of the experimental acre at each farm, and sampled as described previously, before treatments had been applied in 1931. The situation of the three farms was shown in the map in the former paper (7).

The effects of certain physical and chemical treatments on biological activity in these selected soils have been described in the preceding paper in this series. The results from those experiments are used in more detail in this paper for a consideration of seasonal changes in microbial activity in samples taken at intervals of six weeks from any one place during the cropping seasons of two years, and from one of the places for three years. The numbers of bacteria and actinomycetes were estimated by means of Thornton's mannitol-asparagine medium, and the biological activity in samples was determined by the evolution of carbon dioxide. The average values from the plots started in 1931, at the four sampling periods of the two years, are shown in Tables II and III, and in Figs. 1-4. The values relating to the plots receiving limestone

TABLE II*

NUMBERS OF BACTERIA AND ACTINOMYCETES
(MILLIONS PER GM.)
(Averages of 6 plots of 1931)

Brome County					
1931			1932		
April	28	15.313	April	26	11.724
June	9	9.983	June	7	12.201
July	21	11.356	July	18	15.182
Sept.	1	18.061	Aug.	31	11.443
Mean of 48 samples		13.681	12.637		
Compton County					
1931			1932		
May	13	17.199	May	10	7.047
June	23	10.307	June	21	9.158
Aug.	4	12.083	Aug.	2	16.855
Sept.	15	12.878	Sept.	13	7.458
Mean of 48 samples		13.116	10.130		
Sherbrooke County					
1931			1932		
May	28	11.460	May	24	22.786
July	6	11.883	July	5	21.705
Aug.	18	12.457	Aug.	16	22.740
Sept.	29	19.960	Sept.	27	15.259
Mean of 48 samples		13.940	20.643		

Differences required for significance between two values:

Intra-annual 4.406 millions per gm.
Inter-annual 1.981 millions per gm.

*Significantly different values between sampling periods within a year or between the average for each year, are indicated by bold-face type.

TABLE III

EVOLUTION OF CARBON DIOXIDE
(MG. PER 100 GM. PER 100 HR.)
(Averages of 5 plots of 1931)

Brome County					
1931			1932		
April	28	25.68	April	26	17.01
June	9	28.84	June	7	18.91
July	21	31.50	July	18	21.95
Sept.	1	28.62	Aug.	31	17.73
Mean of 40 samples		28.57	18.90		
Compton County					
1931			1932		
May	13	26.77	May	10	25.04
June	23	32.82	June	21	22.95
Aug.	4	38.31	Aug.	2	21.67
Sept.	15	37.44	Sept.	13	15.01
Mean of 40 samples		33.84	21.17		
Sherbrooke County					
1931			1932		
May	28	28.57	May	24	60.83
July	6	22.60	July	5	35.09
Aug.	18	30.69	Aug.	16	42.68
Sept.	29	29.41	Sept.	27	32.63
Mean of 40 samples		27.82	42.81		

Differences required for significance between two values:

Intra-annual 8.475 mg. carbon dioxide
Inter-annual 4.237 mg. carbon dioxide

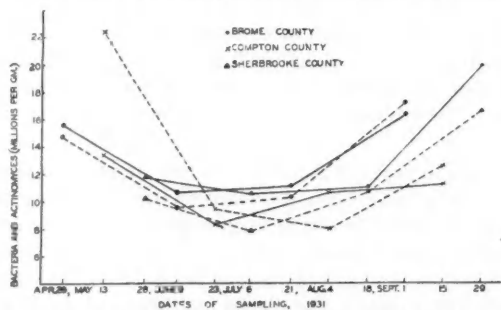


FIG. 1. Seasonal changes in numbers of micro-organisms in 1931. Normal ploughing, solid line; deep ploughing, broken line.

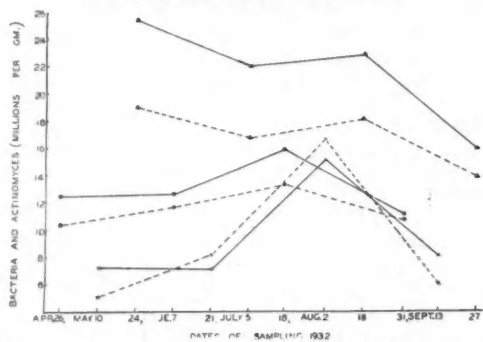


FIG. 2. Seasonal changes in numbers of micro-organisms in 1932. See Fig. 1 for notations.

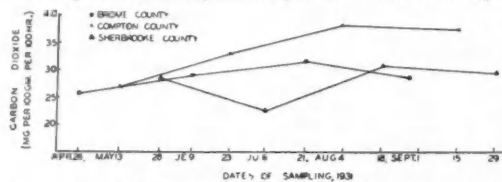


FIG. 3. Seasonal changes in evolution of carbon dioxide in 1931.

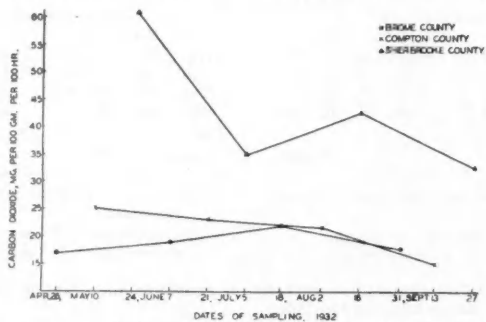


FIG. 4. Seasonal changes in evolution of carbon dioxide in 1932.

have not been included in the tables for carbon dioxide, for the reason stated in the previous paper. The significance of a change between one date of sampling and the next, at any one place, has been based on the standard deviations obtained from the analyses of variance in the two biological factors, as previously determined (7).

The results obtained from the small round plots started in 1932 at the same three places, and continued at the Compton County farm in 1933, are shown in Tables IV and V, and expressed graphically in Figs. 5-8; values from the untreated plots have been compared with the average values of the alkali-treated plots. In view of the small size of the plots and the limited scope of these experiments, no attempt has been made to determine the significance of a change between any one date of sampling and the next.

A first consideration in regard to such biological determinations has been to ascertain the reliability of the values obtained. In regard to the numbers of bacteria (including actinomycetes) the reliability of the mean value of colonies in from two to five plates was ascertained by use of χ^2 ; by this analysis it was found that most of the values obtained (about 80% of 455 available plat-

TABLE IV
INTRA-ANNUAL FLUCTUATIONS IN MICROBIAL ACTIVITY
(Experiments of 1932)

Date of sampling	Bacteria and actinomycetes, (millions per gm.)		Carbon dioxide, (mg. per 100 gm. per 100 hr.)	
	Control plot*	Treated plots**	Control plot*	Treated plots**
<i>Brome County</i>				
April 26	6.02*	—	51.0*	—
June 7	16.50	45.01	44.5	63.8
July 18	41.77	53.85	60.9	74.0
Aug. 31	12.72	25.49	41.5	46.1
<i>Sherbrooke County</i>				
May 24	19.29	—	34.0	—
July 5	13.78	27.02	31.3	46.1
Aug. 16	21.17	35.85	40.7	52.1
Sept. 27	22.80	33.73	31.7	37.4

*Mean of six plots at first sampling.

**Mean of eleven plots having different treatments of CaO, NaOH, Na₂CO₃.

TABLE V
SEASONAL FLUCTUATIONS IN MICROBIAL ACTIVITY
(Compton County—Experiment of 1932)

Date of sampling	Bacteria and actinomycetes, (millions per gm.)		Carbon dioxide, (mg. per 100 gm. per 100 hr.)	
	Control plot*	Treated plots**	Control plot*	Treated plots**
<i>1932</i>				
May 10	4.56*	—	27.6*	—
June 21	8.12	43.05	18.9	39.1
Aug. 2	10.92	35.72	34.8	34.5
Sept. 13	5.71	21.56	19.7	30.0
Average	8.25	33.40	24.5	41.2
<i>1933</i>				
May 18†	10.35	22.17	18.9	35.0
June 13	32.00	39.67	32.9	31.2
July 25	10.35	30.06	36.8	45.8
Aug. 31	16.38	29.18	29.7	37.7
Average	17.27	30.27	29.6	37.4

*Mean values from six plots.

**Mean values from eleven plots treated with CaO, NaOH, Na₂CO₃.

†In Figs. 5-8 this date is shown, by mistake, as May 30.

ings) have been based on reliable samples. It may also be stated that in nearly all cases in which the χ^2 value was found to be excessive or subnormal the numbers of organisms calculated from these plates were not greatly different from the numbers calculated for the parallel samples taken at the same time. The analyses for carbon dioxide were based on readings from single composite samples, usually of 500 gm. each, the gas evolved having

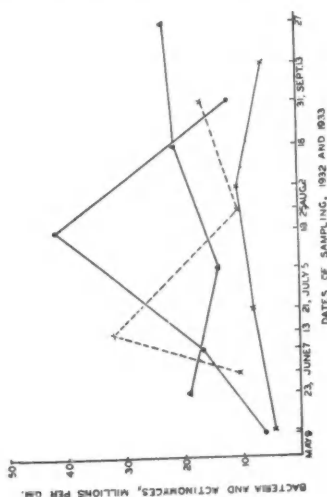


FIG. 5. Seasonal changes in numbers of micro-organisms in alkali-treated plots of 1932. For notations see Fig. 5.

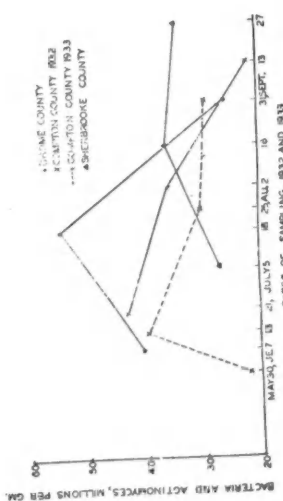


FIG. 6. Seasonal changes in numbers of micro-organisms in untreated plots of 1932. For notations see Fig. 5.

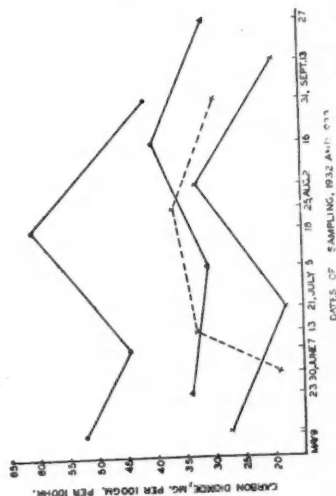


FIG. 7. Seasonal changes in evolution of carbon dioxide in alkali-treated plots of 1932. For notations see Fig. 5.

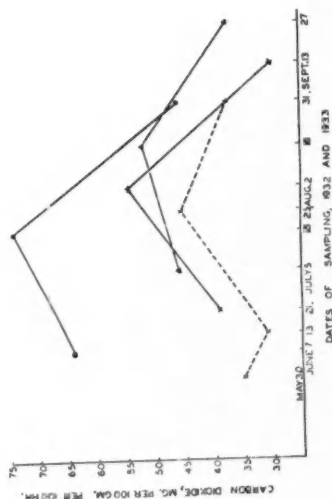


FIG. 8. Seasonal changes in evolution of carbon dioxide in untreated plots of 1932. For notations see Fig. 5.

been collected at intervals during an average aeration time of about 310 hr. The interval between collecting the soil sample in the field and starting the aeration into the train of baryta tubes was rarely greater than 24 hr.

Discussion of Results

In the results obtained from the experiments started in 1931 to test the effects of single fertilizers and limestone, the existence of a significant change in numbers or activity between one date and the next has been indicated by bold-face type in Tables II and III. If however, the results be considered without relation to the statistical analyses but only in regard to the general trend of events, they would seem to indicate that changes in these biological factors occurred regularly in the same direction during a season at all of the places. In regard to bacterial numbers, the results for 1931 confirm the general view of other workers, namely, that numbers were lowest during the summer. In 1932, however, numbers tended to rise towards the end of summer, to be followed by reductions of considerable extent. Since deep ploughing was shown to cause significant reductions in numbers and biological activity, it is interesting to note that the fluctuations shown here occurred to about the same extent, on the whole, in both the deeply ploughed and the normally ploughed plots; the results for numbers of micro-organisms from the two treatments (averages of 5 plots, excluding those treated with limestone) are shown graphically in Figs. 1 and 2. In both years the changes in numbers in the two half-acres were in the same directions, though in some cases at considerably different levels.

The activity of the whole micro-population as determined by the evolution of carbon dioxide, from the experiments started in 1931, offers little evidence of significant differences, such changes occurring only twice in 1932, in the Sherbrooke soil. There appears, however, to be some agreement in the curves for the Brome and Compton soils, in both years; activity in these two soils differs in the two years, the curves for 1932 being almost reverse images of those for 1931. In the case of the Sherbrooke soil the changes in the two years are somewhat similar, though at significantly different levels. The changes in carbon dioxide in the Sherbrooke soil, also, in 1932, resemble the changes in bacterial numbers; otherwise the curves for the two factors do not seem to be related.

There appears to be a definite relation between bacterial numbers and carbon dioxide, when the average values from the three places for the two years are considered. As shown in Tables II and III, the higher or lower numbers in 1932 are associated with corresponding higher or lower amounts of carbon dioxide; these differences, with the exception of the lower numbers of organisms in the Brome soil in 1932, were found to be significant.

It may be noted here that the exclusion from Table II of the results from the plots treated with limestone, the effects of which might be thought to have considerably masked any intra-annual changes, would make no difference to the conclusions stated above.

The results from the experiments started in 1932, to test the effects of certain alkali treatments, seem to indicate a fair amount of agreement in the direction of the changes, at two of the places in regard to numbers, and at all three places in regard to general activity (see Tables IV and V, and Figs. 5-8). It is interesting to compare these results with those obtained from the larger plots situated no great distance away. The small plots at the Compton County farm in 1933 yielded results that are to some extent conformable with the results obtained from the same soil in 1932, especially in regard to the evolution of carbon dioxide. Bacterial numbers appear to be in conformity only in the treated plots; it must be noted, however, that after treatments had been applied in 1932, the results from the control plots are based on single samples of four cores, while those from the treated plots represent the average of 11 such composite samples; the latter should be, therefore, the better measure of these determinations.

During the course of these experiments, determinations for soil moisture were also made. There was no evidence that the moisture content of the soils affected microbial activity. In regard to soil temperature, for which, as well as for rainfall, almost continuous daily records from the district are available for the three years, non-conformity of the bacterial numbers between years would seem to indicate that soil temperature was not a determinant external factor.

The results from the small plots of 1932 would suggest that seasonal changes in numbers and activity of micro-organisms may be superimposed upon any increases in activity brought about by chemical treatments, even when these were such as to cause highly significant increases in the soil population in individual plots.

Acknowledgment

The authors wish to thank Messrs. E. P. Grant and C. B. Taylor, who have been employed as technicians in this work at different times.

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THE INHERITANCE OF DELAYED GERMINATION IN HYBRIDS OF *AVENA FATUA* AND *A. SATIVA*¹

BY L. P. V. JOHNSON²

Abstract

The inheritance of germinability was studied in reciprocal crosses between *Avena fatua*, the seeds of which are non-germinable for several months after harvesting, and *A. sativa*, the seeds of which are fully germinable shortly after harvesting. The following conclusions were drawn from studies of the first three hybrid generations: (1) Germinability is genetically dominant over non-germinability (delayed germination). (2) There is strong evidence that germinability is inherited on the basis of three factors of more or less equal potency, one of which is linked with the factor for grain type. (3) At the time of testing, embryos having completely recessive genotypes were non-germinable; embryos having genotypes with one dominant allelomorph were non-germinable, except in rare instances; embryos having genotypes with two dominant allelomorphs (of the same or different factors) were to a very great extent germinable; embryos having genotypes with three or more dominant allelomorphs were germinable. (4) The germinative potentialities of genotypes vary with the time elapsing between harvesting and testing. (5) There is some evidence that the linked factor has a greater germinative potency than either of the non-linked factors.

Introduction

Studies have been made on the inheritance of the character of delayed germination in reciprocal crosses between the common wild oat, *Avena fatua* L. and a cultivated form, *A. sativa* L., variety Victory. The *A. fatua* parent possesses the property of delayed germination, that is, its seeds are not germinable until several months after harvesting; the seeds of the *A. sativa* parent, on the other hand, are germinable very soon after harvesting. Physiological studies on delayed germination in *A. fatua* have been previously reported (5).

As far as is known, only one previous attempt has been made to study the inheritance of delayed germination, although the phenomenon is by no means of rare occurrence among the species of seed plants. In 1923, Garber and Quisenberry (3) reported data on the inheritance of delayed germination in crosses between *A. sativa* and *A. fatua*, but did not attempt to establish a genetic basis for the character. They concluded from their observations "that in crosses between *A. sativa* and *A. fatua* delayed germination is an inherited recessive character and that it is somewhat loosely linked with the *fatua* type of seed articulation." This statement embodies, essentially, the whole genetic knowledge of delayed germination existing previous to the present work.

¹ Manuscript received August 6, 1935.

Part of a thesis submitted to the faculty of the State College of Washington in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted June 10, 1935. Published as Scientific Paper No. 321, College of Agriculture and Experiment Station, State College of Washington, Pullman, Washington.

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Materials and Methods

Reciprocal crosses were made between *A. fatua* and *A. sativa* var. Victory at the University of Alberta, Edmonton, Alberta, Canada, in 1932. Four hybrid seeds were produced which were designated as follows: Cross 8, in which *A. fatua* was the maternal parent; and Crosses 9, 10, and 11, in which *A. sativa* was the maternal parent.

The *A. fatua* parents were random selections from a plot grown especially for crossing work from a seed stock known to possess a high degree of delayed germination. The seeds from which the plot was grown germinated in consequence of having undergone 18 months of after-ripening. Subsequent germination tests of bulked seed from the crossing plot gave 0.0 and 1.5% germination at four and six months after harvesting.

The *A. sativa* parents were likewise random selections from a plot grown especially for crossing work. Bulk seed from this plot tested two months after harvesting gave 100% germination.

The F_1 plants, together with parental material, were grown in the greenhouse under artificial illumination during the winter of 1932-1933.

The seeds from the F_1 plants were sown in the field in May 1933, about three months after harvesting. Only large, perfectly sound, primary grains were used. A very carefully prepared seed bed together with a period of fine weather provided very favorable conditions for germination. Seeds from the plants of both parents, grown with the F_1 , in the greenhouse, were sown as checks. Daily notes were taken on the seedling emergence of all material.

In anticipation of the non-germinability of the *A. fatua* check, a plot was sown from the original *A. fatua* seed stock (30 months old) to provide check material. At the time of harvesting the F_2 plants, a number of plants were selected from this plot to be used as checks for the F_2 germination tests.

The F_2 plants were harvested in August 1933, placed in open envelopes, allowed to dry in the laboratory for about ten days, then boxed and shipped to Pullman, Washington, where the material was stored in a laboratory at room temperature (about 21° C.).

Germination tests of F_2 and F_3 plants were made in a thermostat-controlled, electrically heated germinator held at 20° C. Fifty seeds were tested from each plant, only large, mature, sound, primary grains being selected. Folded paper pads placed on large blotters which fitted the germinator trays were used for all tests. These pads were cut from white paper towels to a measurement of $2 \times 3\frac{3}{4}$ inches, a size which accommodated 50 seeds without crowding and permitted the placing of 40 pads upon each germinator tray. The F_2 tests were continued for 14 days and the F_3 tests for ten days, although germination was usually completed in eight days. The water used for moistening the pads was kept in the germinator in order to avoid modifying the temperature conditions of the seeds at the time of watering.

The F_3 was grown at Pullman, Washington, in 1934. A complete generation was grown directly from the seeds of F_2 plants of Cross 8 which were tested at the beginning of March. Sprouted seeds were removed daily from the

germinator pads and transplanted to the field. However, in consequence of their genetic constitution, a large number of seeds failed to germinate. It was necessary that these seeds be forced to germinate in order that complete F_3 lines might be grown. This was accomplished by a method which had been previously devised especially for this purpose. After natural germination had ceased, the non-germinated seeds were removed from the germinators, dried thoroughly, scraped over the embryo with a razor, moistened, and returned to the germinator. After this treatment, practically 100% germination occurred. In all, 7,133 seeds out of a total of 7,150 germinated either naturally or by artificial stimulation and were transplanted to the field.

The fact that F_3 lines arising from these transplantings averaged only slightly more than 40 plants each, and the fact that 40 germination pads was the maximum number accommodated by a single germinator tray, led to the limiting of tests of F_3 lines to the seeds of 40 plants.

In both the F_2 and the F_3 , all plants studied for germinability were also classified with respect to seed type in order that linkage relationships might be studied.

In all, individual germination tests were made upon 317 F_2 plants and approximately 3300 F_3 plants comprising 84 families.

Experimental Results and Their Analysis

In studying the inheritance of delayed germination we are dealing, primarily, with generations of embryos rather than mature-plant generations. This fact may lead to some confusion, especially since germinability of the embryo is correlated with a mature-plant character and as the experimental results are largely discussed from the point of view of this correlation. It might be well, therefore, to discuss the matter of generation designations before taking up the discussion of experimental results.

The original hybrid seeds possessed F_1 embryos, seeds of F_1 plants possessed F_2 embryos, seeds of F_2 plants possessed F_3 embryos, etc. Thus, when placed under germinative conditions, the seeds of F_1 plants expressed F_2 segregation for embryonic germinability, the seeds of F_2 plants expressed F_3 segregation, etc. In the genetic analyses, however, the average germinability of the seeds of an F_2 plant, for example, is assumed to be equal to the genetic germinative potentiality of the F_2 embryo carried by the progenitorial seed of the F_1 plant. Thus, when the term F_2 is used, the question of whether an embryo or a mature plant is meant need not arise, for in the analyses the absolute germinability of the embryo and the average germinability of the seeds of the plant derived from the embryo are taken to mean exactly the same thing. Similarly, in the case of the term F_3 . We may look upon the embryo and the plant developed from it as the same individual in different stages of development.

THE FIRST GENERATION

The first generation is represented by the embryos developed directly from the fertilizations brought about by hybridization. Four hybrid seeds (F_1 embryos) were produced. *A. fatua* was the maternal parent in one cross,

the hybrid line from which has been called Cross 8; *A. sativa* was the maternal parent in three crosses, the hybrid lines from which have been called Crosses 9, 10, and 11.

In germination tests made three months after harvesting, all four hybrid seeds germinated, the seeds of the *A. sativa* parent gave practically 100% germination, while the seeds of the *A. fatua* parent failed to germinate. These results clearly indicate that delayed germination is genetically recessive to the *A. sativa* germinative capacity.

THE SECOND GENERATION

The genetic analysis of the F_2 has two bases: (i) the direct observation of the germination or non-germination of F_2 embryos in tests of the seeds of F_1 plants, and (ii) the average percentage germination of F_2 embryos carried by the seeds of individual F_2 plants. The data compiled from tests of the seeds of F_1 plants will be considered first.

1. Results from the Seeds of F_1 Plants

Seeds of the four F_1 plants, together with seeds from both parents, were sown in the field approximately three months after maturity. Only large, mature, sound, primary grains were used. Daily observations on the emergence of seedlings were made. The results from these observations are summarized in Table I, in which the criterion of seed germination is considered to be the emergence of the seedling.

TABLE I
EMERGENCE DATA FROM SEEDS OF F_1 AND PARENTAL PLANTS

Cross	No. of seeds sown	No. of seeds germinated	No. of seeds non-germinated	Ratio, germinated: non-germinated
Cross 8	152	145	7	20.7 : 1
Cross 9	91	90	1	90.0 : 1
Cross 10	94	90	4	22.5 : 1
Cross 11	137	136	1	136.0 : 1
Total of all crosses	474	461	13	35.5 : 1
<i>A. fatua</i> parent	50	0	50	—
<i>A. sativa</i> parent	50	50	0	—

The observed ratio between the numbers of germinated and non-germinated seeds varies greatly in the different crosses. This variation is, apparently, not correlated with the reciprocal nature of the crosses. None of the observed ratios show a close correspondence with any standard F_2 ratio. Furthermore, the wide deviations among the different observed ratios rule out attempts to fit these ratios, as they now stand, to a hypothetical F_2 ratio.

2. Results from the Seeds of Individual F_2 Plants

The seeds of individual F_2 plants of Crosses 8, 9, and 10 were tested for germinability. The time elapsing between harvesting and testing was three months for Cross 9, five months for Cross 10, and six months for Cross 8. The results of these tests are combined and summarized in Table II.

TABLE II
FREQUENCY DISTRIBUTION OF PERCENTAGE GERMINATION OF INDIVIDUAL *F₂* PLANTS OF CROSSES 8, 9, AND 10

[illegible]

The F_2 plants of these crosses were also classified with respect to type of grain. It has been shown by Aamodt, Johnson and Manson (1) that in crosses between *A. fatua* and *A. sativa* segregation occurs for three grain types, namely, *sativa*, intermediate, and *fatua* types, in the monohybrid ratio of 1:2:1. As in the previous work, *sativa* types are designated by w, intermediate types by I, and *fatua* types by W. (See Fig. 1). In Table II, the data from germination tests of each cross have been divided on the basis of segregation for these grain types.

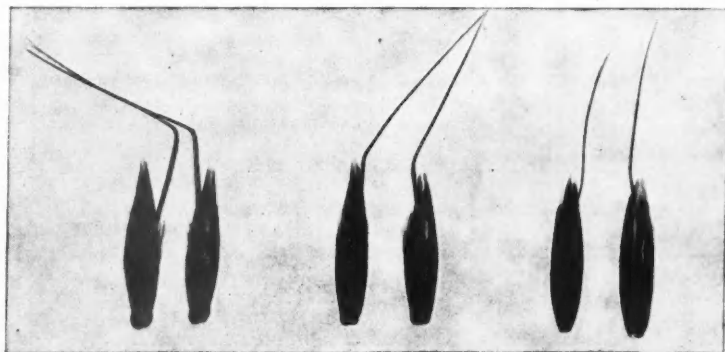


FIG. 1. Segregation for grain type. Left to right in pairs: *fatua* (W), intermediate (I), and *sativa* (w) types.

Though tested at different periods the germination percentages of the F_2 plants of the different crosses are distributed in essentially the same manner and with similar relative frequencies in the various classes. It seems reasonable to assume, therefore, that at the time of testing, the germinative capabilities of the various genotypes were essentially the same in all crosses. There is no reason to suspect that there was any appreciable germination of seeds possessing the genetic constitution of the non-germinable parent, especially as only one germination occurred among the 700 seeds comprising the 14 *A. fatua* checks. This germination occurred in one of the checks of the Cross 8 tests which was tested six months after harvesting. In view of the above facts, the data from all three crosses are considered to be directly comparable, despite their compilation at different times.

The non-occurrence of types possessing delayed germination (0% class in Table II) is satisfactorily accounted for by the assumption that the F_2 recessive embryos, carried by the seeds of the F_1 plants, failed to germinate, thus eliminating non-germinable types from the population of F_2 plants.

The correlation between high degree of germinability and *sativa* (w) type of grain is very marked. There also appears to be a tendency for *fatua* (W) types to be less germinable than intermediate (I) types. The correlation between germinability and grain type, calculated by the coefficient of correlation method, is as follows: $r = .307 \pm .037$ for Cross 8, $r = .385 \pm .062$ for Cross 9, and $r = .086 \pm .071$ for Cross 10.

The nature of the genetic linkage relationship between germinability and grain type may be deduced very simply. It is known definitely that type of grain is inherited on the basis of a single gene or a single gene complex (1). Thus, a linkage relationship with grain type can concern only a single chromosome. Therefore, it may be assumed that one genetic factor for germinability (AA , Aa , or aa) is linked with the factor for grain type (WW , Ww , or ww) in the following manner: $(Wa)(Wa)$, giving the *fatua* type; $(Wa)(wA)$, giving the intermediate type; and $(wA)(wA)$, giving the *saliva* type. It is obvious that there must be at least one factor for germinability in addition to the linked factor, for otherwise all *fatua* types would be non-germinable*.

In the genetic analyses, the observed F_2 data were compared with a series of frequencies calculated on the basis of several theoretical ratios derived from different assumptions regarding numbers and germinative capacities of factors. Alternative assumptions were made regarding the theoretical germinative capacities of genotypes consisting of one heterozygous and a number of recessive factors for germinability ($A_1a_1a_2a_2$ or $a_1a_1A_2a_2$ in the case of two factors; $A_1a_1a_2a_2a_3a_3$ or $a_1a_1A_2a_2a_3a_3$ or $a_1a_1a_2a_2A_3a_3$, in the case of three factors, etc.)**. The single heterozygous factor (Aa , for short) was assumed to be germinable in certain cases and to be non-germinable in others, the alternative assumptions giving markedly different theoretical ratios. An example of the method of calculating these ratios is provided by Table VIII. The theoretical ratios derived from the different assumptions are given in Table III.

The various phenotypic classes are designated by the grain-type symbols (w , I , or W) followed by a number expressing the theoretical germination percentage to the nearest whole number.

It might be useful to illustrate the method used in deriving the phenotypes given in Table III. For example, in the case of the 189 ($A_1a_1A_2a_2A_3a_3$) type of F_2 plant in Assumption 4, the theoretical germinability of the F_3 embryos is 57 germinable (i.e., 16 Aw , 30 AI , 11 AW) and seven non-germinable (i.e., 2 al , 5 aW) which gives an average of 89.10% germinability. (Refer to Table VIII.) Thus, in formulating the phenotypes, the grain type of each F_2 plant was associated with the average theoretical percentage germination of the F_3 embryos borne by the seeds of the plant in question.

To facilitate the comparison of the various theoretical ratios given in Table III with the observed data given in Table II, theoretical frequencies have been calculated for Crosses 8 and 9 and are presented in Table IV. The frequencies for Cross 10 were not calculated because the population of this cross, (88), is practically the same as that of Cross 9, (86).

*The terms germinable and non-germinable are used in this paper to refer, respectively, to ability and inability of seeds to germinate under the conditions of the test in question. Germinability is used as a general term referring to germinative capacities, whether positive or negative. These terms make unnecessary the use of such unwieldy expressions as delayed germination, immediate germination, etc.

**Henceforth in this paper the condition of a single heterozygous factor and a series of homozygous recessive factors for germinability will be expressed by the symbol Aa .

TABLE III
THEORETICAL F_2 RATIOS BASED ON VARIOUS ASSUMED NUMBERS AND GERMINATIVE CAPACITIES OF FACTORS

Assumption no.	No. of factor pairs	Genotype of <i>A. fatua</i>	Genotype of <i>A. sativa</i>	Germinative capacity of <i>Aa</i>	Calculated ratios based on seeds of F_2 plants
1	2	(<i>Wa</i> ₁)(<i>Wa</i> ₁) <i>a</i> ₂ <i>a</i> ₂	(<i>wA</i> ₁)(<i>wA</i> ₁) <i>A</i> ₂ <i>A</i> ₂	germinable	4(<i>w</i> 100) : 2(<i>I</i> 100) : 1(<i>W</i> 100) : 4(<i>I</i> 94) : 2(<i>I</i> 75) : 2(<i>W</i> 75) : 1(<i>W</i> 0)
2	2	(<i>Wa</i> ₁)(<i>Wa</i> ₁) <i>a</i> ₂ <i>a</i> ₂	(<i>wA</i> ₁)(<i>wA</i> ₁) <i>A</i> ₂ <i>A</i> ₂	non-germinable	4(<i>w</i> 100) : 2(<i>I</i> 100) : 1(<i>W</i> 100) : 4(<i>I</i> 69) : 2(<i>I</i> 25) : 2(<i>W</i> 25) : 1(<i>W</i> 0)
3	3	(<i>Wa</i> ₁)(<i>Wa</i> ₁) <i>a</i> ₂ <i>a</i> ₂ <i>a</i> ₂	(<i>wA</i> ₁)(<i>wA</i> ₁) <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂	germinable	16(<i>w</i> 100) : 14(<i>I</i> 100) : 7(<i>W</i> 100) : 8(<i>I</i> 98) : 8(<i>I</i> 94) : 4(<i>W</i> 94) : 2(<i>I</i> 75) : 4(<i>W</i> 75) : 1(<i>W</i> 0)
4	3	(<i>Wa</i> ₁)(<i>Wa</i> ₁) <i>a</i> ₂ <i>a</i> ₂ <i>a</i> ₂	(<i>wA</i> ₁)(<i>wA</i> ₁) <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂	non-germinable	16(<i>w</i> 100) : 14(<i>I</i> 100) : 7(<i>W</i> 100) : 8(<i>I</i> 89) : 8(<i>I</i> 69) : 4(<i>W</i> 69) : 2(<i>I</i> 25) : 4(<i>W</i> 25) : 1(<i>W</i> 0)
5	4	(<i>Wa</i> ₁)(<i>Wa</i> ₁) <i>a</i> ₂ <i>a</i> ₂ <i>a</i> ₂ <i>a</i> ₂	(<i>wA</i> ₁)(<i>wA</i> ₁) <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₄ <i>A</i> ₄	germinable	64(<i>w</i> 100) : 74(<i>I</i> 100) : 37(<i>W</i> 100) : 16(<i>I</i> 99) : 24(<i>I</i> 98) : 8(<i>W</i> 98) : 12(<i>I</i> 94) : 12(<i>W</i> 94) : 2(<i>I</i> 75) : 6(<i>W</i> 75) : 1(<i>W</i> 0)
6	4	(<i>Wa</i> ₁)(<i>Wa</i> ₁) <i>a</i> ₂ <i>a</i> ₂ <i>a</i> ₂ <i>a</i> ₂	(<i>wA</i> ₁)(<i>wA</i> ₁) <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₂ <i>A</i> ₄ <i>A</i> ₄	non-germinable	64(<i>w</i> 100) : 74(<i>I</i> 100) : 37(<i>W</i> 100) : 16(<i>I</i> 97) : 24(<i>I</i> 89) : 8(<i>W</i> 89) : 12(<i>I</i> 69) : 12(<i>W</i> 69) : 2(<i>I</i> 25) : 6(<i>W</i> 25) : 1(<i>W</i> 0)

Comparison of the theoretical frequency distributions of Table IV with the actual data of Table II brings out the following facts:

(1) Assumptions 1, 3, and 5 give theoretical segregation for a non-germinable type and several types with germinability ranging from 75 to 100%. The observed data deviate greatly from such segregation in that numerous well-distributed individuals occur in the range between the zero and 75% classes. Assumptions 1, 3, and 5 are ruled out, therefore, on the basis of theoretical frequency distributions which are in marked disagreement with the observed data.

(2) Assumption 2 may be eliminated from further consideration on the basis of greatly excessive theoretical frequencies in the case of *I*25 and *W*25 types.

(3) Assumption 6 provides theoretical frequencies which agree much more closely with the observed data than those of any of the assumptions discussed above. However, in the *I* 89-100 and *W* 89-100 classes the theoretical are considerably greater than the observed frequencies, and *vice versa* in the case

TABLE IV
THEORETICAL F_2 FREQUENCIES FOR CROSSES 8 AND 9 CALCULATED ON THE BASIS OF
ASSUMPTIONS GIVEN IN TABLE III

Number of cross	Assump- tion no.	Theoretical frequencies of various phenotypic classes											
		W						I					w
		0	25	69	75	89	94-100	25	69	75	89	94-100	100
8(N=143)	1	8.9	—	—	17.9	—	8.9	—	—	17.9	—	53.7	35.8
	2	8.9	17.9	—	—	—	8.9	17.9	35.8	—	—	17.9	35.8
	3	2.2	—	—	8.9	—	24.5	—	—	4.5	—	67.1	35.8
	4	2.2	8.9	8.9	—	—	15.6	4.5	17.9	—	17.9	31.3	35.8
	5	0.6	—	—	3.4	—	31.8	—	—	1.1	—	70.4	35.8
	6	0.6	3.4	6.7	—	4.5	20.7	1.1	6.7	—	13.4	50.3	35.8
9(N=86)	1	5.4	—	—	10.8	—	5.4	—	—	10.8	—	32.3	21.5
	2	5.4	10.8	—	—	—	5.4	10.8	21.5	—	—	10.8	21.5
	3	1.3	—	—	5.4	—	14.8	—	—	2.7	—	40.4	21.5
	4	1.3	5.4	5.4	—	—	9.4	2.7	10.8	—	10.8	18.8	21.5
	5	0.3	—	—	2.0	—	19.1	—	—	0.7	—	42.3	21.5
	6	0.3	2.0	4.0	—	2.7	12.4	0.7	4.0	—	8.0	30.2	21.5

of the I 69, W 69, I 25 and W 25 classes. The theoretical ratios of assumption 6 are considered, however, to show a sufficiently close agreement with the observed data to warrant further consideration in goodness-of-fit tests.

(4) Assumption 4 is found to provide theoretical frequencies which are on the whole in good agreement with the observed data, though this agreement is somewhat obscured by the straggling distribution of intermediate classes. The agreement is much better than that of any of the other assumptions discussed. The only considerable deviations between theoretical and observed frequencies occur in the case of W25 types, where the theoretical are much too high in Crosses 8 and 10. These deviations and the straggling nature of the intermediate classes demand special consideration.

It would appear from the above discussion that there is at least a fair possibility of Assumption 4 providing a basis for an explanation of the inheritance of germinability; therefore, the relations between this assumption and the observed data will be discussed in considerable detail.

A point to be settled before taking up this discussion involves the decision of whether the non-germinability of seeds of F_1 plants (see Table I) should be considered as genetic or environmental in nature. A number of facts strongly indicate that non-germinability was for the most part, if not entirely, due to the genetic nature of the seeds in question. In the first place, the seeds were carefully selected for strong vitality (see section on Materials and Methods) and germinative conditions were very favorable. Secondly, 100% germination occurred in the *A. sativa* parental check. And thirdly, a series of several other *A. sativa* varieties (checks in tests for delayed germination in fatuoids), which had been grown in the greenhouse with the F_1 plants, showed practically 100% germination in tests which were directly comparable to those of the seeds of F_1 plants. It is reasoned that the non-germination of seeds of F_1 plants, with possibly one or two exceptions, must be considered as genetic expressions.

Assumption 4 implies that the single heterozygous factor Aa is potentially non-germinable. This being the case, the I 25 and W 25 classes should have been eliminated with the W0 types when the seeds of the F_1 plants were tested. Such eliminations occurred to a large extent, apparently, in the case of W 25 types in Crosses 8 and 10; but not in the case of I 25 types in any of the crosses, nor of W 25 types in Cross 9 (Compare Tables II and IV). It will be noted by reference to Table I that excessive numbers of non-germinated seeds occurred in the case of the F_1 plants of Crosses 8 and 10; further, it may be noted that the degree to which these numbers are in excess of theoretical W0 frequencies corresponds to the degree to which observed W 25 numbers are deficient relative to theoretical frequencies (Compare Tables I, II, and IV). It is significant to note in this connection that in Cross 9, where the W 25 observed corresponds closely to the theoretical frequency, the observed number of non-germinable seeds of the F_1 plant is also in close agreement with the theoretical W0 frequency.

A possible explanation of the fact that F_2 embryos with Aa genotypes germinated in some and not in other cases (see Table I) would be to assume that, at the time of testing the seeds of F_1 plants, these embryos were passing through the critical stage in the after-ripening process where some were barely germinable while others had not quite after-ripened sufficiently to germinate. It is also possible that these variations in germinability may be explained on the basis of different germinative potentialities of the different factors.

It will also be necessary to explain why, in Crosses 8 and 10, W 25 types frequently failed to germinate while I 25 types appeared to germinate in nearly all cases. The explanation lies, in all probability, in the fact that different factors are involved in producing the two types. W 25 types are always produced by a single non-linked heterozygous factor (A_2a_2 or A_3a_3), while I 25 types are always produced by the heterozygous condition of the linked factor (A_1a_1). (See Table VIII.) This explanation assumes that the linked factor is more potent than the non-linked factor, and, therefore, produces the germinable state after a shorter period of after-ripening.

Theoretical frequencies calculated on the basis of Assumptions 4 and 6, respectively, have been tested for goodness-of-fit to the observed data by the method described by Hayes and Garber (4). These tests are summarized in Tables V and VI. With respect to Crosses 8 and 10, the observed frequencies of W 25 classes in each cross and the frequencies of non-germinated seeds of the respective F_1 plants have been grouped into a single class considered to represent the combined frequencies of W0 and W 25 classes for the cross in question. In the case of Cross 9 no groupings were made, the single non-germinable seed of the F_1 plant being considered as the W0 frequency.

The dividing lines in Table II indicate the manner in which the W 25 and I 25 classes were divided from W 69 and I 69 classes, and also how the latter were separated from the W 89-100 and I 89-100 classes. The deviation in the line separating the higher classes, is made in consequence of the additional phenotypes and the greater frequencies in the I 89-100 classes.

In Table V, where the theoretical frequencies are based upon Assumption 4, the goodness-of-fit tests indicate very close correspondence between these frequencies and the observed data in all crosses. The values of P are as

TABLE V
OBSERVED F_2 DATA (INCLUDING NON-GERMINABLE TYPES) OF CROSSES 8, 9 AND 10 COMPARED WITH THEORETICAL FREQUENCIES CALCULATED ON THE BASIS OF ASSUMPTION 4.
VALUES OF χ^2 AND P FOR EACH CROSS ARE ALSO GIVEN.

Phenotypic class	Cross 8		Cross 9		Cross 10	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
w100	39	37.5	23	21.8	22	23.0
W100	19	16.4	12	9.5	15	10.1
I100 } I89 }	50	51.6	32	29.9	33	31.6
I69	16	18.8	8	10.9	10	11.5
W69	12	9.4	6	5.4	6	5.8
I25	4	4.7	1	2.7	2	2.9
W25 } W0 }	10	11.7	4 1	5.4 1.4	4	7.2
N	150	150.1	87	87.0	92	92.1
χ^2	2.0092		3.2575		4.3868	
P	between .90 and .95		between .80 and .90		between .50 and .70	

follows: between .90 and .95 for Cross 8, between .8 and .9 for Cross 9, and between .5 and .7 for Cross 10. The values of P were determined from a table provided by Fisher (2).

In Table VI, where the theoretical frequencies are based upon Assumption 6, the goodness-of-fit tests indicate a very poor correspondence between these

TABLE VI
OBSERVED F_2 DATA (INCLUDING NON-GERMINABLE TYPES) OF CROSSES 8, 9 AND 10 COMPARED WITH THEORETICAL FREQUENCIES CALCULATED ON THE BASIS OF ASSUMPTION 6.
VALUES OF χ^2 AND P FOR EACH CROSS ARE ALSO GIVEN

Phenotypic class	Cross 8		Cross 9		Cross 10	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
w100	39	37.5	23	21.8	22	23.0
W100 } W89 }	19	26.4	12	15.3	15	16.2
I100 } I97 }	50	66.8	32	38.8	33	41.0
I89	16	7.0	8	4.1	10	4.3
W69	12	7.0	6	4.1	6	4.3
I25	4	1.2	1	0.7	2	0.7
W25 } W0 }	10	4.1	4 1	2.0 0.3	4	2.2
N	150	150.0	87	87.1	92	91.7
χ^2	36.5256		10.3219		13.8083	
P	< .01		between .10 and .20		between .02 and .05	

frequencies and the observed data of all three crosses. The values of P are as follows: less than .01 for Cross 8, between .1 and .2 for Cross 9, and between .02 and .05 for Cross 10.

The results of these goodness-of-fit tests serve to eliminate Assumption 6 from further consideration, and to establish Assumption 4 as the basis of a tentative explanation of inheritance in the F_2 .

The question of the possibility of genetic explanations other than those already studied should receive some attention. Studies revealed that as the number of factors was increased above three, the frequencies of the highly germinative phenotypic classes increased at the expense of the frequencies of the lower germinative classes, rendering theoretical frequencies more and more unlike the observed data. Nor was it possible to justify, on the basis of observed data, assumptions regarding germinative capacities of factors such as, for example, the non-germinability of AA and $AaAa$ types*. After a thorough examination of alternative explanations, it is concluded that Assumption 4 provides, as far as can be determined, the only possible basis of explaining the observed data.

The significance of the P values calculated in Table V should be examined from the point of view of the justifiability of the divisions of phenotypic classes made in Table II. The positions of the dividing lines in Table II are based upon natural, though not always very distinct, divisions in the frequency distribution. The line dividing the W 25 and I 25 classes from the W 69 and I 69 classes runs through a natural break in the distribution. The line separating the W 100 class from the W 69 class is determined by the range shown in the w 100 class, which is a natural group, as well as by points of low frequency in the distribution. The line dividing the I 89 and I 100 classes from the I 69 is extended beyond the lines of the w 100 and W 100 classes in order to include the natural range of these combined classes. It will be noted that this line, like all other lines, runs through points of low frequency on either side of which the frequencies increase. It is felt that the natural distribution of the data given in Table II justifies the divisions made between phenotypic classes.

The straggling nature of the distribution of the intermediate classes (I 69 and W 69) should, if possible, be accounted for. In considering this matter it should be borne in mind that the variability in the observed germination percentages may be due to either or both of two things: the occurrence of different degrees of non-germination among types theoretically expected to germinate, and the chance deviations from the theoretical segregation ratios. (Each frequency represents the average germination percentage of a segregating line of F_2 embryos). Variability due to these two causes is sufficient to explain the range of germinability in the intermediate classes of Cross 9, but in the case of Crosses 8 and 10 it appears probable that still another factor influenced variability. It may be that, owing to internal or external

*Genotypes are simplified by the omission of homozygous recessive factors.

conditions affecting the material of Crosses 8 and 10 at the time of testing, the $AaAa$ and AA types were in some cases non-germinable. Theoretically, the non-germinability of these types would transform I 69 and W 69 classes into I 44 and W 44 classes, respectively. This explanation would also account for the occurrence of two w types of intermediate germinability*. There is also a possibility that differences in the germinative potentialities of the different factors may explain some of these variations in germinability.

The analysis and discussion of F_2 data has been carried to a point where it is possible to state a definite hypothesis in explanation of the inheritance of germinability. The hypothesis is, briefly, as follows: Germinability is inherited as a dominant character on the basis of three factors of essentially equal potency, one of which is linked with the factor for grain type; the embryos having genotypes with a single heterozygous factor for germinability (Aa) are potentially non-germinable under the conditions of the tests, but may be germinable in relatively rare instances; the embryos having genotypes with one homozygous (AA) or two heterozygous ($AaAa$) factors for germinability are potentially germinable under the conditions of the tests, but may be non-germinable in relatively rare instances; with the exception of the triple recessive all other genotypes are potentially germinable.

The validity of this hypothesis will be examined further in the light of F_3 data.

THE THIRD GENERATION

It has been shown that the seeds of F_3 plants possess F_4 embryos, therefore, the result of a germination test of the seeds of an F_3 plant represents the average germinability of a complete line of F_4 embryos. The averages of germinability of F_4 lines represent the germinative potentialities of the respective F_3 genotypes.

Sixty-six lines of F_3 plants from Cross 8 and 18 from Cross 10 were tested for germinability. These tests were continued over a period extending from two and a half to five months after harvesting, which was within the range between the time when the *A. sativa* parent was completely germinable and the time when the *A. fatua* parent began to show germinability. This period of testing was very similar, with respect to time after harvesting, to the time of the tests of F_2 plants.

The coefficient of correlation for the percentage germination obtained in the F_2 tests and the average percentage of germination of the plants of F_3 lines was $.517 \pm .061$ for Cross 8 and $.760 \pm .67$ for Cross 10.

Environmental variations in germinability resulting from differences in the period of after-ripening given the various F_3 lines prior to testing have made impossible the brief summarization of the F_3 data in tabular form. Consequently, it has been necessary to limit the presentation of the F_3 results to general descriptions of breeding behavior in which the lines from each class of F_2 phenotypes have been considered as a separate group.

*The w plant of lowest germinability gave 79% germination when tested in the F_3 .

Table VII was prepared with the view of simplifying the discussion of relations between observed and theoretical frequencies in the F_3 segregation for germinability and grain types. The theoretical F_3 ratios given in this table were derived by averaging the theoretical germination percentages

TABLE VII
 F_3 RATIOS EXPECTED FROM THE VARIOUS F_2 TYPES ON THE BASIS OF THE HYPOTHESIS.
THE NUMBER OF TESTED F_3 LINES FROM EACH F_2 TYPE IS ALSO GIVEN

F_2 type	No. of F_3 lines tested	Theoretical F_3 ratios
w100	16	w100
I100	14	I100
I89	12	16(w100) : 14(I100) : 8(I89) : 8(I69) : 2(I25) : 7(W100) : 4(W69) : 4(W25) : 1(W0)
I69	15	4(w100) : 2(I100) : 4(I69) : 2(I25) : 1(W100) : 2(W25) : 1(W0)
I25	5	1(w100) : 2(I25) : 1(W0)
W100	5	W100
W69	13	7(W100) : 4(W69) : 4(W25) : 1(W0)
W25	4	1(W100) : 2(W25) : 1(W0)
W0	0	W0
Total.....	84	

expected in the F_4 (embryonic generation) lines from each F_3 grain type, and then relating these averages directly to the respective F_3 grain types (see Table VIII).

The F_3 data are, on the whole, in good agreement with the theoretical frequencies expected on the basis of the hypothesis. However, many F_3 lines tended to show much lower percentages of germination than would be expected theoretically, the occurrence of which appeared to be correlated with early testing with respect to all grain types but particularly in the case of W types.

Germinability of F_3 lines from w F_2 types varied from 50 to 99.3%. Distinctly low germinability was shown by only three lines, each of which was tested between two and a half and three and a half months after harvesting. The remainder of the lines tested during this period showed reasonably high germinability (up to 94%). These results may be explained by assuming that w types arising from F_2 types having genotypically only two or three dominant allelomorphs for germinability(AA or AAAa) were incapable of high germination in early tests, while w types possessing four to six dominant allelomorphs were relatively highly germinable at this time. The average germination of all w lines tested was 84%, while with the three low-germinating lines omitted it was 91%.

Two F_3 lines from I 100 F_2 types showed relatively low average germination percentages, both being among the lines tested in the early part of the testing period. These two lines are presumed to have arisen from F_2 types having genotypically only three dominant allelomorphs (AaAA). The average

germination of all I 100 F_3 lines tested was 86%, while with the two lines of lower germination omitted it was 90%.

Four out of the five F_3 lines from W 100 F_2 types were tested between three and three and a half months after harvesting and gave germination percentages between 32 and 71 with an average of 49%; the remaining line was tested five months after harvesting and gave 89% germination. These facts indicate that at least one of the non-linked factors is somewhat less potent than the linked factor, thus requiring a longer period of after-ripening to produce the germinable state. (W types never possess either of the dominant allelomorphs of the linked factor).

In most cases the observed data of F_3 lines from I 89 F_2 types were in close agreement with the theoretical frequencies, values of P running as high as .95. In some cases, however, the values of P were as low as .01 owing, for the most part, to very low observed frequencies in the W 100 and W 69 classes and to very high frequencies in the W 25 and W 0 classes. The occurrence of these lines with a lower degree of germination than was expected, appeared to be correlated with early testing. Early testing may be expected to cause $AaAa$ and AA types to tend toward non-germinability, particularly when these types concern the less potent non-linked factors, upon which W types must depend for germinability. This would tend to shift W 100 and W 69 classes toward the W 25 class and to shift the W 25 class toward the W 0 class. Granting that the observed deviations may be explained in this way, the data of F_3 lines from I 89 F_2 types may be said to be in close correspondence with the theoretical frequencies.

The discussion applied to the F_3 lines from I 89 F_2 types applies essentially to the F_3 lines from I 69 F_2 types. The values of P for the latter lines range from .8 to .05.

The observed data of the F_3 lines from I 25 F_2 types showed very close correspondence with the theoretical ratio. Only one of these lines was tested less than four months after harvesting. This line, tested three months after harvesting, showed a tendency toward low germinability in both w and I segregates; early testing, of course, could not reduce the germinability of the W segregates, they being of the W 0 type.

In the case of F_3 lines from W 69 F_2 types, six showed good correspondence between observed and theoretical frequencies, while seven showed a deficiency of observed frequencies in the W 100 and W 69 classes with an excess in the W 25 and W 0 classes. The occurrence of lines with lower degrees of germination than the expected was correlated with early testing. An explanation of these deviations from theoretical frequencies has been given in connection with the discussion of lines from I 89 F_2 types.

The F_3 lines from W 25 F_2 types were all tested less than three and a half months after harvesting, and all show a high deficiency of observed frequencies in the W 100 class and a great excess of frequencies in the W 0 class. These deviations from theoretical expectations may be explained on the basis of the non-germinability of AA types due to premature testing.

In no case did the F_3 results make it necessary to change the classification of an F_2 plant. However, in certain instances the restricted germination which occurred in certain early-tested lines made it impossible definitely to associate F_3 segregation with F_2 types.

It would appear, judging from the tendency toward low germinability observed in F_3 lines tested about three months after harvesting, that a longer period of after-ripening was required to render AA and $AaAa$ types germinable in F_3 plants grown in Washington than was required in F_2 plants grown in Alberta; for, in the case of F_2 plants of Cross 9, tested three months after harvesting, there was little indication of non-germinability of AA and $AaAa$ types.

A further test of the hypothesis was made by marking in a number of F_3 rows the point in the row up to which the seedlings from readily germinable F_2 seeds were transplanted, and later observing the grain type of the plants on either side of the mark. F_3 lines from four I 25 and six I 69 F_2 types were studied in this manner.

In a line from an I 25-type F_2 plant which gave very nearly 25% germination, the plants from readily germinable seeds were practically all of the w type, while the plants from artificially germinated seeds were all of the I and W types. This indicates that AA genotypes were germinable while Aa and aa were non-germinable. However, in a line from an F_2 plant which gave considerably more than 25% germination, I- as well as w-type plants were produced from readily germinable seeds, indicating that the high germination percentage was at least partly due to the germination of a number of Aa type embryos. Conversely, in a line from an F_2 plant which gave far less than 25% germination several w plants were produced from artificially germinable seeds, indicating that the low germination percentage was in this case at least partly due to the non-germination of a number of AA type embryos.

In F_3 lines from I 69 F_2 types it was found that readily germinable seeds produced w, I and W plants in about the expected proportion (4w : 6I : 1W), and the plants from artificially stimulated seeds also occurred in about the expected proportion (2I : 3W).

These observations on the grain type of plants produced from naturally and artificially germinated seeds, which, however, represent at best only a partial elucidation of F_3 segregation, afforded in every case excellent support to the hypothesis.

The coefficients of correlation for grain type and percentage germination in F_3 lines from I-type F_2 plants ranged from $.000 \pm .114$ in the case of certain I 100 types to $.904 \pm .21$ in the case of certain I 25 types.

Granting certain assumptions which are believed to be justified, none of the experimental results of the F_3 can be cited as definite evidence against the validity of the hypothesis based on the F_2 ; but, on the other hand, many data afford support to the hypothesis. The F_3 results showed even more clearly than the F_2 that one factor for germinability is linked with the factor for grain type, and they supported the assumption that there are two non-

TABLE VIII
INHERITANCE SCHEME BASED UPON ASSUMPTION 4 (SEE TABLE III)
 $(wA_1)(wa_1)A_2A_3A_4 \times (Wa_1)(W_2a_1)a_2a_3a_4$
(*A. sativa*)
 F_1 $(wA_1)(Wa_1)A_2A_3A_4$

F_1 genotypes	F_2 phenotypes*	F_2 phenotypes	F_2 Av. % germ.	F_2 phenotypes	F_2 Av. % germ.
1 $(wA_1)(wa_1)A_2A_3A_4$	Aw	Aw	100	Aw	100
2 $(wA_1)(wa_1)A_2A_3A_4$	Aw	Aw	100	Aw	100
2 $(wA_1)(wa_1)A_2A_3A_4$	Aw	Aw	100	Aw	100
2 $(wA_1)(Wa_1)A_2A_3A_4$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
4 $(wA_1)(wa_1)A_2a_3A_4$	Aw	Aw	100	Aw	100
4 $(wA_1)(Wa_1)A_2a_3A_4$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
4 $(wA_1)(Wa_1)A_2a_3A_4$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
8 $(wA_1)(Wa_1)A_2a_3A_4$	AI	16Aw : 30AI : 2AI : 11AW : 3aW	89.10	16(Aw) : 14(1Aw : 2AI : 1AW) : 2(1Aw : 2AI : 1aW) : 8(16Aw : 30AI : 2AI : 11AW : 5aW) : 7(AW) : 1(aW) : 8(4Aw : 6AI : 2AI : 1AW : 3aW) : 4(11AW : 5aW) : 4(3aW : 1AW)	84.18
1 $(wA_1)(wa_1)A_2a_3a_4$	Aw	Aw	100	Aw	100
2 $(wA_1)(wa_1)A_2a_3a_4$	Aw	Aw	100	Aw	100
2 $(wA_1)(Wa_1)A_2a_3a_4$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
4 $(wA_1)(Wa_1)A_2a_3a_4$	AI	4Aw : 6AI : 2AI : 1AW : 3aW	68.75	4(Aw) : 2(1Aw : 2AI : 1AW) : 2(1Aw : 2AI : 1aW) : 4(4Aw : 6AI : 2AI : 1AW : 3aW) : 1(AW) : 2(3aW : 1AW) : 1(aW)	67.19
1 $(wA_1)(wa_1)a_2a_3A_4$	Aw	Aw	100	Aw	100
2 $(wA_1)(wa_1)a_2a_3A_4$	Aw	Aw	100	Aw	100
2 $(wA_1)(Wa_1)a_2a_3A_4$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
4 $(wA_1)(Wa_1)a_2a_3A_4$	AI	4Aw : 6AI : 2AI : 1AW : 3aW	68.75	4(Aw) : 2(1Aw : 2AI : 1AW) : 2(1Aw : 2AI : 1aW) : 4(4Aw : 6AI : 2AI : 1AW : 3aW) : 1(AW) : 2(3aW : 1AW) : 1(aW)	67.19
1 $(Wa_1)(Wa_1)A_2A_3A_4$	AW	AW	100	AW	100
2 $(Wa_1)(Wa_1)A_2A_3A_4$	AW	AW	100	AW	100
4 $(Wa_1)(Wa_1)A_2a_3A_4$	AW	11AW : 5aW	68.75	7(AW) : 4(11AW : 5aW) : 4(3aW : 1AW) : 1(aW)	67.19

TABLE VIII—Concluded

INHERITANCE SCHEME BASED UPON ASSUMPTION 4 (SEE TABLE III)

$(wA_1)(wA_1)A_2A_2A_3 \times (W_{a1})(W_{a1})a_2a_2a_3$
(A. sativa) *(A. fatua)*

F_1 $(wA_1)(W_{a1})A_2a_2A_3$

F_2 genotypes	F_2 phenotypes	F_2 phenotypes	F_2 Av. % germ.	F_2 phenotypes	F_2 Av. % germ.
1 $(wA_1)(wA_1)a_2a_2a_3$	Aw	Aw	100	Aw	100
2 $(wA_1)(W_{a1})a_2a_2a_3$	Al	1Aw : 2al : 1aW	25.00	1(Aw) : 2(1Aw : 2al : 1aW) : 1(aW)	37.50
1 $(W_{a1})(W_{a1})A_2A_2a_3a_3$	AW	AW	100	AW	100
2 $(W_{a1})(W_{a1})A_2a_2a_3a_3$	aW	3aW : 1AW	25.00	1(AW) : 2(1AW : 3aW) : 1(aW)	37.50
1 $(W_{a1})(W_{a1})a_2a_2A_3A_3$	AW	AW	100	AW	100
2 $(W_{a1})(W_{a1})a_2a_2A_3a_3$	aW	3aW : 1AW	25.00	1(AW) : 2(1AW : 3aW) : 1(aW)	37.50
1 $(W_{a1})(W_{a1})a_2a_2a_3a_3$	aW	aW	0.00	aW	0.00
F_2 ratio: 16(w100) : 14(1100) : 7(W100) : 8(189) : 8(W69) : 2(125) : 4(W25) : 1(W0)					

*In phenotypic symbols, A and a denote germinability and non-germinability, respectively; w, l, and W denote sativa, intermediate and fatua grain types, respectively.

linked factors for germinability. It was shown in the F_3 that the balance between germinability and non-germinability in embryos with Aa , AA , or $AaAa$ genotypes is somewhat unstable and may be influenced by relatively small variations in the state of after-ripening, a fact which lends justification to certain assumptions made in explaining irregularities in F_2 results. The F_3 results also served to establish on a firmer basis the probability, suggested somewhat vaguely by the F_2 , that the linked factor possesses more potent germinative capacities than do the non-linked factors.

However, the occurrence of many deviations from the theoretically expected results, while perhaps satisfactorily explained, makes it impossible to lay claim to the proving of the hypothesis. It is concluded that F_3 results, while not affording definite proof, strongly indicate that the hypothesis, in its essential features, represents the true explanation of the inheritance of germinability.

General Discussion and Conclusions

The results from the first three hybrid generations of plants have been analyzed and in most respects more or less fully discussed. It now remains to discuss, briefly, certain matters bearing on the analysis as a whole and to draw conclusions regarding the essential nature of the inheritance of germinability.

The data of the second and third generations have been in a general way characterized by a lack of clear-cut distinctions between phenotypic classes, and by a frequent occurrence of irregularities in germinative breeding results. This state of affairs made genetic analysis more difficult and probably less convincing than would have been the case under more favorable circumstances.

The validity of the whole genetic analysis hinges upon the acceptability of the following assumption: that under the conditions of nearly all tests, embryos with Aa genotypes were non-germinable, but that in a very few tests they were germinable; and, that under the conditions of most tests embryos with AA or $AaAa$ genotypes were germinable, but that in a few cases they were non-germinable. This assumption, if granted, would account for the straggling nature of the F_2 and F_3 frequency distributions and for the observed irregularities in breeding results; it would place the observed frequencies in harmony with the theoretical frequencies, and thus serve in establishing the hypothesis.

A number of results afforded definite support to this assumption: (1) The observations on the grain type of plants grown from seeds which germinated naturally and from seeds in which germination was artificially stimulated indicated that embryos with Aa and AA genotypes could be either germinable, or non-germinable in the same test. (2) It was found in F_3 tests that the type of germinability in which the non-germinability of embryos with Aa , AA or $AaAa$ genotypes is assumed was correlated with early testing. It is inferred that very shortly after harvesting only embryos with six dominant allelomorphs ($AAAAAA$) are germinable, and that as time goes on embryos with a progressively smaller number of dominant allelomorphs become germinable

Thus, at a certain stage embryos with Aa , AA , $AaAa$ or triple recessive genotypes will be non-germinable; later, AA and $AaAa$ types will be germinable; still later the Aa types will germinate; and, finally, the triple recessive ($A. fatua$) types will germinate. Of course, it is to be expected that there would be considerable overlapping of the periods when germinability occurs in the successive types. This line of reasoning is in accord with the assumption under discussion.

Evidence was provided by the F_3 which indicated that at least one of the non-linked factors possessed less potent germinative capacities than the linked factor, and thus was capable of producing germinability only after a longer period of after-ripening than was required by the linked factor. However, this difference in potency is not considered great enough to receive special consideration in the general statement of inheritance.

Crossing over has not been discussed because of the fact that the data are not suitable for its study; the presence of two non-linked factors for germinability would, in most cases, obscure its expression; and, in instances where the non-linked factors are in a recessive condition, the variation in germinability of A_1A_1 and A_1a_1 genotypes would make it difficult or impossible to establish its occurrence.

Since the F_3 results have not altered the essential nature of the hypothesis based on the F_2 , which has been adequately discussed, it is believed unnecessary to carry the general discussion further.

It is concluded that the experimental results, as analyzed, give very strong indication that the mode of inheritance of germinability in the hybrids of *A. fatua* and *A. sativa* is, in its essential features, as follows: Germinability is dominant over non-germinability (delayed germination) and is inherited on the basis of three factors of more or less similar germinative potencies, one of which is linked with the factor for grain type. Under the conditions of the tests, embryos with Aa genotypes were in nearly all cases non-germinable but in certain relatively rare cases were germinable; embryos with AA or $AaAa$ genotypes were to a very great extent germinable but in a few instances were non-germinable; embryos with recessive genotypes were non-germinable; embryos having genotypes with three or more dominant allelomorphs were germinable.

This mode of inheritance presumes the following parental genotypes:

A. sativa, $(wA_1)(wA_1)A_2A_2A_3A_3$

A. fatua, $(Wa_1)(Wa_1)a_2a_2a_3a_3$

Assuming these genotypes, the F_2 ratio from the cross between these two species is approximately, 16(w100) : 14(I100) : 7(W100) : 8(I89) : 8(I69) : 4(W69) : 2(I25) : 4(W25) : 1(W0).

Acknowledgments

The writer is indebted to Dr. E. F. Gaines and Dr. H. F. Clements for helpful suggestions in connection with the preparation of the manuscript, and to Mr. Lewis Weiner for excellent routine assistance.

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CHARACTER COMBINATIONS IN RELATION TO ENDOSPERM DEVELOPMENT IN INTER-SPECIFIC TRITICUM CROSSES¹

By J. B. HARRINGTON²

Abstract

A study was made of the frequency of different character combinations in relation to seed plumpness in the F_2 generation of crosses of *T. durum*, *T. dicoccum* and *T. persicum* with *T. vulgare*. Some inter-specific character combinations occurred frequently and others were rare, which was expected since the characters varied markedly in their frequency of inter-specific combinations with other characters. The data did not support the popular assumption that inter-specific character combinations of value were to be found largely in plants from shrunken seeds rather than in those from plump seeds. Plants from plump seeds possessed nearly as large a proportion of the combinations which were missing in the plants from shrunken seeds as did the latter plants with respect to the former. The difference could not be considered significant and none of the missing combinations appeared to be important as far as practical breeding is concerned.

Introduction

Marked success has been achieved in producing desirable character combinations from crosses between wheats of different chromosome numbers. Examples of this success are found in the work of Hayes *et al.* (4), McFadden (5), Harrington (1), A. A. Sapehin (7) and L. A. Sapehin (8).

In recent studies made by Harrington (2) and Harrington and Marshall (3) on crosses between *vulgare* and three 14-chromosome species, it was demonstrated that a definite relation existed between the degree of development attained by the F_2 seed endosperm and various morphologic characters of the F_2 plant. In general, the most shrunken seeds produced the most *vulgare*-like plants although many shrunken seeds did not produce *vulgare*-like plants and many plump seeds did not produce plants resembling the 14-chromosome parental variety. Desirable *vulgare*-like plants were obtainable from each plumpness class, the largest proportion being from the shrunken seeds. In the material studied, certain character combinations were relatively frequent, others were infrequent and some did not appear at all. It was apparent that the differences were not all casual and therefore that some combinations might be rarely or perhaps never obtained.

A study was made, therefore, of the frequency of different character combinations in relation to seed plumpness for each of the crosses, Iumillo (*durum*) \times Marquis (*vulgare*), Vernal (*dicoccum*) \times Marquis, and Marquis \times Black Persian (*persicum*). The present paper gives the results of this study. The description of parents, description of hybrids and general analysis of the relations between seed plumpness and various morphological characters are presented fully in the paper by Harrington and Marshall (3).

¹ Manuscript received September 16, 1935.

Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta. The results were reported in full at the Meeting of the Associate Committee on Field Crop Diseases at Winnipeg on April 4, 1935.

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It was shown in the paper by Harrington and Marshall (3) that the *vulgare*-like plants from plump seeds were more *vulgare*-like than the *vulgare*-like plants from shrunken seeds in the IM cross (Iumillo, (*T. durum*) × Marquis, (*T. vulgare*)) but the opposite was true in the VM cross (Vernal, (*T. dicoccum*) × Marquis, (*T. vulgare*)) and the MP cross (Marquis, (*T. vulgare*) × Black Persian, (*T. persicum*)) gave results resembling those of the VM cross. The question then arose, were *all* of the *vulgare*-like plants from shrunken seeds of the IM cross less *vulgare*-like than those from plump seeds, and, if not, were they consistently unlike *vulgare* in certain characters? Similarly it was desirable to ascertain whether the *vulgare*-like plants from plump seeds of the VM and MP crosses were consistently unlike the *vulgare*-like plants from shrunken seeds in certain characters.

TABLE I
DESCRIPTION OF THE *vulgare*-LIKE PLANTS FROM PLUMP F_2 SEED OF THE THREE CROSSES

Cross	Plant number	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character	Rachis articulation	Awning	Glume adherence
IM	4	I	V	IV	V	V	V	IV	I	IV	ID	I	V	IV			
	12	V	V	V	V	V	V	V	V	V	V	I	V	V			
	13	V	V	I	V	V	V	V	IV	I	D	I	V	V			
	15	D	D	IV	IV	V	D	V	I	IV	V	IV	V	V			
	16	IV	V	IV	V	V	V	V	V	D	V	IV	I	IV			
	17	I	V	IV	V	V	V	V	V	ID	V	ID	IV	ID			
	22	V	V	IV	I	IV	V	V	V	V	IV	I	V	IV			
	27	IV	V	V	V	V	V	V	V	V	V	IV	V	V			
	29	IV	V	IV	I	V	V	V	V	I	ID	I	V	IV			
	37	I	IV	ID	I	V	V	V	IV	ID	D	IV	V	IV			
	44	I	I	IV	D	IV	V	V	V	V	V	V	V	IV			
	49	I	V	IV	V	V	IV	V	ID	V	V	V	IV	ID			
	51	V	V	V	V	IV	V	V	V	V	V	IV	V	IV			
	58	V	V	V	V	V	IV	V	V	V	V	V	V	ID			
	62	I	IV	I	ID	I	D	V	V	IV	V	IV	V	V			
	71	V	IV	V	I	V	V	V	V	V	V	V	V	V			
	72	IV	V	V	V	V	V	V	V	V	V	V	V	IV			
	73	IV	V	I	I	IV	V	V	V	V	V	V	I	V			
	74	V	V	V	IV	V	V	IV	I	I	D	IV	I	ID			
	83	ID	IV	IV	IV	V	V	V	V	V	I	IV	V	IV			
	85	D	V	I	I	IV	V	V	V	V	D	IV	V	ID			
MP	2			V	D	V	V	ID	V		ID						
	5			ID	ID	V	V	D	IV		I						
	24			I	V	IV	V	I	ID		ID						
	35			IV	I	IV	V	V	V		V						
	47			IV	I	V	V	IV	D		V						
VM	3	V	V	I	ID	IV	V	D	ID	IV					V	D	V
	9	I	V	I	IV	I	IV	I	ID	V					D	V	D
	17	ID	D	IV	IV	V	I	IV	IV	ID					D	V	D
	45	IV	V	IV	IV	V	I	D	D	V					V	D	V

NOTE:—V = resembling *vulgare* closely; D = resembling 14-chromosome species closely; I = intermediate between V and D; IV = intermediate between V and I; ID = intermediate between D and I. Character resemblances to the 14-chromosome species are italicized.

vulgare-like plants from plump seeds (see Table I). The IM section of the table shows that although there were fewer *vulgare*-like plants from shrunken seeds (18) than from plump seeds (21), the former showed a sprinkling of D or ID for *all* of the three characters under consideration. The MP section shows D or ID for two of the characters concerned but none of the plants had *persicum* resemblance for rachis width. The VM section of the table shows a large proportion of D and ID for rachis hairiness but no D or ID for the other two characters.

Durum-vulgare Character Relationships

The discovery that the *vulgare*-like plants from plump seeds were neither D nor ID for three characters in each cross immediately brought up the question of the frequency of the various interspecific character relations. As both the IM and the VM crosses were being used in a breeding program they were utilized for a study of this question. Random lots of F_2 plants grown from plump and from shrunken seeds of each cross were examined and their degree of resemblance to the parental varieties was recorded for each of a number of morphologic characters. The various character relations were analyzed, first on the basis of both V and IV indicating *vulgare*-ness and both D and ID representing resemblance to the 14-chromosome parent, and finally on the basis of combinations of V with D, only. The preliminary analysis of the population from plump seeds of the Iumillo \times Marquis cross is given in Table III.

The table was prepared as follows: Plant No. 1 was considered for the relation between spike form and each of the twelve other characters.

TABLE III

THE FREQUENCY DISTRIBUTION OF COMBINATIONS OF V OR IV WITH D OR ID FOR ALL POSSIBLE TWO-CHARACTER RELATIONS IN THE 102 *durum* \times *vulgare* F_2 PLANTS FROM *plump* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character
Spike form													
Spike compactness	7												
Stem hollowness	5	7											
Stem thickness	15	21	13										
Rachis width	18	20	16	12									
Rachis hairiness	9	11	13	19	17								
Shoulder width	13	12	10	19	13	14							
Glume l-w ratio	11	19	11	20	18	20	11						
Keel sharpness	10	17	16	21	24	19	24	25					
Beak character	25	37	27	26	23	37	31	25	29				
Keel prominence	11	13	13	25	21	14	17	18	11	29			
Brush character	17	22	18	18	14	15	18	17	21	20			
Seed character	13	20	17	22	20	19	15	10	20	24	21	18	
Average	13	17	14	19	18	17	16	17	20	28	18	18	18

This plant was ID for spike form. It was V or IV for rachis width and three other characters. In each case a mark was made in the square where the line or column for spike form met the column or line for the other character, following to some degree the method used in filling in an ordinary correlation surface. The next step was to consider the spike compactness of Plant No. 1 in its relations, and so on for all the characters of Plant No. 1. Each of the 101 other plants was considered in the same way. To prevent duplication of numbers throughout the table all relations were taken from D or ID to V or IV.

Table III demonstrates that *all* of the different two-character combinations of V or IV with D or ID occurred in this random population of only 102 F_2 plants. Beak character showed a high average number of combinations. Stem hollowiness and spike form showed only half as many combinations. The other characters were more or less intermediate in this respect.

TABLE IV

COMBINATIONS OF V (*vulgareness*) WITH D (*durumness*) FOR ALL POSSIBLE TWO-CHARACTER RELATIONS IN THE 102 *durum* \times *vulgare* F_2 PLANTS FROM *plump* SEEDS

Character	Spike form	Spike compactness	Stem hollowiness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character
Spike form													
Spike compactness	2												
Stem hollowiness	0	0											
Stem thickness	7	11	4										
Rachis width	6	7	0	1									
Rachis hairiness	4	5	2	10	4								
Shoulder width	3	3	0	8	3	4							
Glume l-w ratio	2	7	2	10	2	6	2						
Keel sharpness	2	6	3	8	4	5	5	2					
Beak character	13	21	8	10	10	20	11	8	12				
Keel prominence	0	3	2	10	3	3	3	2	7				
Brush character	7	10	5	6	4	4	6	6	4	9	5		
Seed character	1	2	0	2	1	4	1	0	0	3	2	1	
Averages	3.9	6.5	2.2	7.3	3.8	5.9	4.1	4.2	4.4	11.0	3.6	5.6	1.4

If, instead of taking V and IV to represent *vulgareness* and D and ID to represent *durumness*, only combinations of V with D, that is, full resemblances to the parental species, are taken into account, relatively few combinations are obtained. The results for all possible two-character combinations of V and D in the Iumillo \times Marquis plants from plump seeds are given in Table IV. The numbers in the table are small, as would be expected. In eight cases no plant had the combination. Such a result could occur by chance. However, seven of these eight cases occurred with respect to the relationships of only two characters, stem hollowiness and seed character, with the other characters. It is evident that for some character pairs combinations of V with D occurred much less frequently than they did for other character pairs.

TABLE V

COMBINATIONS OF V (*vulgareness*) WITH D (*durumness*) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN THE 55 *durum* × *vulgare* F₂ PLANTS FROM *shrunk* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character
Spike form	0												
Spike compactness	0	1											
Stem hollowness	3	6	1										
Stem thickness	0	1	0	1									
Rachis width	4	7	4	9	2								
Rachis hairiness	1	1	1	1	1	3							
Shoulder width	4	9	0	2	0	4	1						
Glume l-w ratio	1	2	1	4	1	6	1	5					
Keel sharpness	8	15	3	10	6	10	3	4	9				
Beak character	0	1	1	4	2	5	1	3	0	6			
Keel prominence	4	4	3	6	3	6	2	6	3	7	1		
Brush character	1	1	0	1	0	2	1	0	2	2	0	4	
Seed character													
Averages	2.2	4.0	1.3	4.0	1.4	5.2	1.4	3.2	2.9	6.9	2.0	4.1	1.2

The results on two-character combinations of D and V in the plants from shrunk seeds appear in Table V. Here we find the non-appearance of 12 combinations. The total population was only 55 plants or roughly half of that of the plants from plump seeds, consequently the percentage of absent combinations is lower in the plants from shrunk seeds. Here as in Table IV the distribution of zeros in the table is not fortuitous. Seven of the 11 cases occurred with respect to the relationships of stem hollowness and seed character with the other characters, as was the case in the results in Table IV. Four of the 11 misses occur in the spike form column. Going back to Table IV we find two of the eight misses in the spike form column. The relative frequencies of combinations in Tables IV and V are very similar, the lowest frequencies being for stem hollowness and seed character and the highest for beak character in both sets of figures. There appears to be no essential difference between the populations from shrunk and plump seeds with respect to the frequency of occurrence of different character combinations.

Dicoccum-vulgare Character Relationships

Data on the cross Vernal emmer × Marquis were studied in the same manner as for the *durum* × *vulgare* cross. Before discussing the character combinations in plants from shrunk and from plump seeds, it is of interest to examine the combinations in a random population without regard to endosperm development. Table VI shows the combinations of V (*vulgareness*) with D (*dicoccumness*) for all possible two-character relationships in a random population of 276 F₂ plants. All combinations were represented excepting one (seed character with spike form) and it is probable that the absence of that combination is a matter of chance, since several other combinations are represented only once.

TABLE VI
THE FREQUENCY DISTRIBUTION OF THE COMBINATIONS OF V (*vulgareness*) WITH D (*dicoccumness*) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN A RANDOM POPULATION OF 276 F_2 PLANTS OF THE CROSS VERNAL EMMER \times MARQUIS

Character	Rust reaction	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Spikelet adherence	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Shoulder shape	Glume l-w ratio	Keel sharpness	Awning	Seed character
Field rust reaction	6															
Spike form	40	11														
Spike compactness	15	3	9													
Stem hollowness	7	4	14	8												
Rachis articulation	30	6	61	14	8											
Spikelet adherence	21	7	11	2	13	20										
Rachis width	44	9	21	4	12	21	2									
Stem thickness	31	6	40	12	4	1	18	16								
Glume adherence	19	6	10	5	14	39	13	31	25							
Rachis hairiness	25	14	19	11	18	42	6	14	36	14						
Shoulder width	53	13	43	17	18	18	11	9	7	37	27					
Shoulder shape	18	4	25	4	10	26	1	8	21	14	3	21				
Glume l-w ratio	47	12	31	12	11	15	13	14	5	31	41	19	25			
Keel sharpness	71	35	61	30	21	43	25	17	32	46	36	27	29	38		
Awning	1	0	25	2	3	7	8	10	6	12	13	17	3	17	25	
Seed character																
Averages	27	9	26	9	10	22	11	14	16	20	20	21	13	21	34	10

Table VII gives the frequency distribution for the various two-character combinations of D with V which were found in a random population of 102 F_2 plants from plump seed of the VM cross. There were 21 missing combinations of which 11 involved spike form and stem thickness, but none involved keel sharpness and awning, and only one was in the spike compactness column.

TABLE VII
COMBINATIONS OF V (*vulgareness*) WITH D (*dicoccumness*) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN THE 46 *dicoccum* \times *vulgare* F_2 PLANTS FROM *plump* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Awning
Spike form	1											
Spike compactness	0	0										
Stem hollowness	0	2	0									
Rachis articulation	0	1	0	1								
Rachis width	0	2	1	0	0							
Stem thickness	2	4	2	0	3	1						
Glume adherence	0	1	0	0	0	1	3					
Rachis hairiness	1	3	0	2	1	0	3	1				
Shoulder width	0	2	0	1	1	0	1	0	0			
Glume l-w ratio	2	7	3	2	2	2	6	6	6	3		
Keel sharpness	4	17	10	6	6	2	4	11	8	7	7	
Awning												
Averages	0.8	3.3	1.3	1.2	1.3	0.8	2.1	1.9	2.1	1.3	3.5	6.8

In Table VIII, results are given for the plants from shrunken seeds. There were 18 missing combinations, instead of about 24 which would be expected on the basis of the results in Table VII. Another difference between the results in Tables VII and VIII is the greater frequency of combinations in-

TABLE VIII

COMBINATIONS OF V (*vulgareness*) WITH D (*dicoccumness*) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN THE 39 *dicoccum* × *vulgare* F_2 PLANTS FROM *shrunken* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Awning
Spike form	1											
Spike compactness	1	3										
Stem hollowness	0	2	1									
Rachis articulation	1	4	1	1								
Rachis width	1	7	1	0	0							
Stem thickness	0	5	1	0	1	1						
Glume adherence	1	3	2	1	0	4	4					
Rachis hairiness	0	2	1	0	0	2	2	0				
Shoulder width	1	4	1	1	0	0	0	3	1			
Glume l-w ratio	0	2	2	0	1	0	0	3	2	1		
Keel sharpness	0	2	2	0	1	0	0	3	2		1	
Awning	2	5	4	2	3	2	0	4	1	2		2.2
Averages	0.7	3.2	1.5	0.7	1.0	1.5	1.3	2.1	0.9	1.3	1.0	

volving glume adherence, shoulder width, keel sharpness and awning, in the material from plump seeds, and of combinations with stem thickness in the plants from shrunken seeds. Considering the 18 misses, they involved principally the characters, spike form, stem thickness, shoulder width and glume length-width ratio, but not spike compactness; and only one was concerned with awning. The populations from plump and shrunken seeds may be said to agree less well, as to the frequency of the different character combinations, than did the comparable populations of the IM cross.

Discussion of Results

From the plant breeding standpoint it is important to know, first, if certain character combinations are unobtainable or only rarely found in F_2 populations of inter-specific wheat crosses and, second, whether the lack of these character combinations is linked up with the degree of development of the F_2 seeds.

Frequency of Inter-specific Character Combinations

The most extensive data on the frequency of different D-V character combinations appears in Table VI where the results on 276 F_2 plants of the cross Vernal emmer × Marquis are given. The various combinations showed a wide range in the frequency of their occurrence, seven appearing less than

three times each and five others being represented more than 46 times each. The following tabulation gives the frequency distribution of all the combinations:

	Combination frequencies								
	0	1-2	3-5	6-9	10-15	16-23	24-34	35-46	47 up
Number of combinations	1	6	11	17	28	20	18	14	5

Some combinations occurred frequently and others rarely. On the whole, the combinations which occurred most frequently, such as the keel sharpness of one species with the rust reaction of the other, or the spikelet adherence of one species with the spike compactness of the other, are usually considered to be fully as unusual inter-specific combinations as those which appeared rarely, such as the keel sharpness of one species with the glume adherence of the other.

Some characters showed a relatively small tendency to combine inter-specifically with others, namely, spike form and stem hollowness, whereas other characters showed a large tendency toward such combinations, *viz.*: spike compactness, spikelet adherence and rust reaction.

One combination which is economically desirable, that of stem rust resistance with *vulgare* seed character, occurred only once in the whole population. It is easy to see that to add various other characters to this combination might require a very large population.

In practical plant breeding, some of the characters studied in the VM cross could be ignored in selection work as it is unimportant whether they be present in the V, D, or an intermediate condition. Examples are rachis hairiness, shoulder shape and awning. Comparatively few of the characters are necessary to have in the D or V condition. Exceptions are rust reaction and seed character, respectively. The varying frequency of combinations of *vulgare*-ness with *dicoccum*ness for different character pairs should, therefore, be regarded as a guide to the breeder rather than as a detriment to his work. Where he desires combinations which are infrequent, he should use very large populations from which to select.

It is of interest to note that some character combinations were absent from both groups of plants studied in the Iumillo \times Marquis cross. The total number of plants was 157 and five combinations were missing. Inspection of the two sets of data shows three combinations which are represented only once in each population. A further random reduction in the size of the populations would be expected to change some of these frequencies of one to misses, and an increase in the sizes of the populations would, by the same reasoning, supply some of the missing combinations. This expectation is fortified by the fact that the distribution for frequency of combination is

normal for each group of plants, the only real difference being the natural one that the distribution for the 102 plants from plump seeds has a higher mean than the one for the 55 plants from shrunken seeds. This is shown by the following tabulation:

	Combination frequencies				
	0	1 and 2	3 to 5	6 to 9	10 and over
Table IV data	8	18	24	16	12
Table V data	12	30	20	13	2

Furthermore, there is no reason to expect that a population of several hundred plants would not fill in the missing combinations, as occurred in the case of the VM random population of 276 plants.

Discussion of the frequency of combinations found in the small VM populations from plump and shrunken seeds is not necessary as the situation is covered by the foregoing discussions on the large random VM population and the IM populations.

Relation of Seed Plumpness to Character Combinations

A detailed study of all possible two-character relations was made in a *durum* \times *vulgare* and a *dicoccum* \times *vulgare* cross to ascertain whether plants from shrunken seeds furnished any two-character combinations not obtainable in plants from plump seeds. In 1930 Thompson (9) concluded that the shrunken seeds in inter-specific wheat crosses were much more valuable to the practical breeder than the plump seeds and should receive special care. In 1934 Harrington and Marshall (3), in a study of three inter-specific wheat crosses, concluded that no special attention need be paid to the shrunken seeds as they did not appear to possess character combinations unobtainable in plants from plump seeds.

The results of the *durum* \times *vulgare* study presented in Tables IV and V show eight combinations missing in the 102 plants from plump seeds and 12 in the 55 plants from shrunken seeds. This difference slightly favors the plants from shrunken seeds (on the basis of 8 misses in 102 plants 15 could be expected in 55 plants) but does not appear to have significance. Any possible significance is detracted from by the fact that the 102 plants from plump seeds furnished 7 combinations not present in the plants from shrunken seeds. (Spike compactness with spike form, rachis width with spike form, glume length-width ratio with stem hollowness, glume length-width ratio with rachis width, seed character with rachis width, keel prominence with keel sharpness and seed character with keel prominence), whereas plants from shrunken seeds furnished only 3 combinations not found in the plants from plump seeds, (stem hollowness with spike compactness, shoulder width with stem hollowness and seed character with keel sharpness).

All of these combinations involve characters of species-differentiating value as has been shown by Percival (6), Watkins (10) and others. Sapehin (7) and Sapehin (8), have shown that it is difficult and probably erroneous to assign more critical species-differentiating value to one of these characters than to others among them as none of them are found exclusively in a given species. Therefore it is reasonable to regard the missing combinations as being of equal importance. The plants from plump seeds actually possessed a larger proportion of combinations missing in the plants from shrunken seeds than did the latter with respect to the former. It follows that the plants from shrunken seeds cannot be said to have supplied combinations unobtainable in the plants from plump seeds.

The *dicoccum* \times *vulgare* results presented in Tables VII and VIII show 21 missing combinations in the 46 plants from plump seeds and only 18 in the 39 plants from shrunken seeds. The difference favors the plants from shrunken seeds and is strengthened by the fact that these plants possessed 15 combinations not shown by the plants from plump seeds, whereas the latter showed only 11 combinations not present in the plants from shrunken seeds. This indicates that, in the *dicoccum* \times *vulgare* cross, plants from shrunken seeds might furnish a larger range of combinations than plants from plump seeds. However, the difference between the two sets of results is small and probably not significant. The absence of some combinations in Tables VII and VIII as well as in Tables IV and V appears to be due to the small size of the populations. In the much larger population reported in Table VI there is only one combination missing.

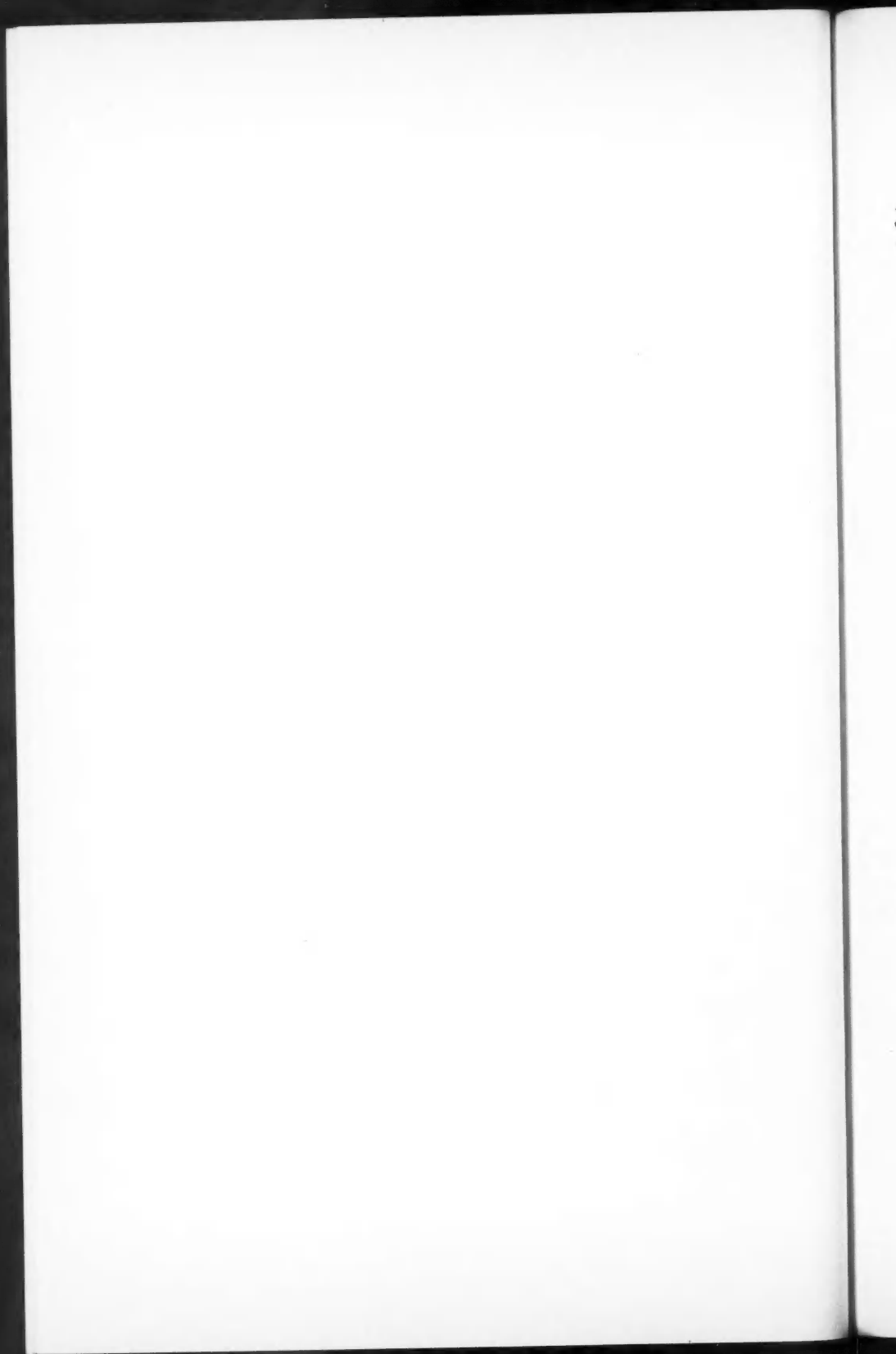
Considering the results on both crosses it may be concluded that there is no evidence to show that F_2 plants from shrunken seeds possess character combinations not obtainable in F_2 plants from plump seeds. In fact, the data suggest that the various two-character combinations are as likely to be obtained from one group of plants as from the other, in either cross.

The results of this study indicate that a sufficiently large population should show all of the possible two-character combinations of V and D. It follows that a larger population would be necessary to reveal all possible three-character combinations of V and D, a still larger population to show all possible four-character combinations, and so on. If it may be assumed that the frequency of occurrence of any given combination of *vulgareness* for certain characters with *dicoccumness* for other characters depends largely upon the size of population, then the results presented in Tables IV, V, VII and VIII indicate no appreciable superiority of plants from shrunken seeds over plants from plump seeds.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 13, SEC. D.

DECEMBER, 1935

NUMBER 6

GROWTH OF *MYA ARENARIA* L. IN THE BAY OF FUNDY REGION¹

BY CURTIS L. NEWCOMBE²

Abstract

Data are presented which demonstrate the validity of "annual rings" as age determinants in *Mya arenaria* L. The time curve of growth for this latitude depicts a relatively rapid rate during the first four years, after which a pronounced decrease obtains. Relatively little variation has been found in the rate of growth characterizing the widely separated parts of the Bay studied. In general, specimens 18 mm. in length grow about 12 mm. (66%) in one year and those 26 mm. grow about 9 mm. (33%). These represent typical values for growth in the Bay of Fundy region under favorable conditions.

The length of the growing season during 1931 was approximately four months, —May to August, inclusive. During 1930, growth continued until about Dec. 1. Such a late fall growth is considered unusual for the Bay of Fundy region.

Data are presented which show that variations in seasonal growth rates during the same and different years correspond with abundance of diatoms and not with temperature. The rate of growth has been found to vary directly with time of submergence. Wide variations in salinity and sulphide content of the beach are required to reduce the amount of growth significantly. Excess surface silt on the beach limits the growth rate and survival time of *Mya arenaria*.

I. Introduction

Comparatively few growth studies of marine pelecypods have been conducted under natural conditions. Mead and Barnes (14), Kellogg (9) and Belding (1) are among the early contributors to our knowledge of the growth of *Mya arenaria* on the east coast of the United States. In view of the lack of information dealing with the growth of this species in the colder and more northern waters of the Bay of Fundy, the present study was undertaken.

The purpose of the investigation has been; first, to determine the validity of periodic rings on the valves for age determination; second, to ascertain the normal rate of growth that is representative of the latitude in question; and third, to evaluate the influence of certain environmental factors on distribution, growth rate and survival by using rate of growth in nature as a criterion. Certain ecological aspects of the problem have received special consideration.

In order to confirm and enlarge the data obtained in the study under natural conditions, experimental boxes were planted on various beaches at different tidal levels, and the specimens placed therein were measured at particular intervals to determine the seasonal and annual rates of growth. In this way, it has been possible to establish the significance of the concentric rings on the shells for age determination. The two principal length classes of *Mya*

¹ Original manuscript received July 8, 1935.

Contribution from the Zoological Laboratories of the University of Toronto, the Chesapeake Biological Laboratory and the University of Maryland; with financial assistance from the National Research Council of Canada.

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arenaria employed are designated "small" and "large" clams. As a consequence of the correspondence obtained between relative growth rate and abundance of plankton organisms present, particular attention has been given to the composition of the food of this lamellibranch.

II. Experimental Methods

The linear dimensions of *Mya arenaria* were measured with a vernier caliper reading to 0.1 mm. The length measurement is the greatest anterior-posterior dimension and the width constitutes the greatest radius with the umbo as a centre. In view of the shape of the valves, this width dimension was accepted rather than a measurement made on a line at right angles to the long axis (Fig. 2, A). Throughout the paper, length is used as a basic index of size. Other indices such as width, thickness, weight of shell and dry weight of body parts have been studied and will be treated in a subsequent paper.

Measurements of "annual rings" for determining age were made with dividers which were transferred to a vernier caliper and read in millimetres, tenths being estimated. On account of the width of the groove constituting the winter check, a vernier measurement does not possess greater accuracy.

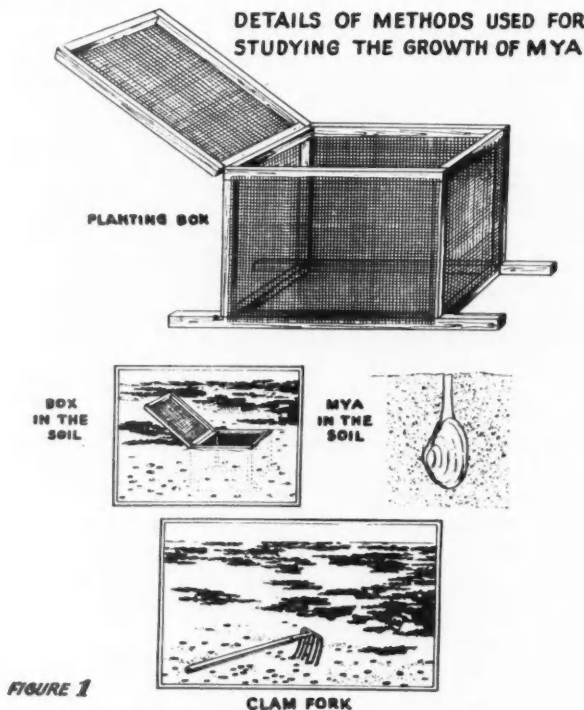


FIG. 1. Showing materials of *Mya* experiments.

Increments of growth in nature under different experimental conditions were determined by planting specimens in experimental boxes placed in the desired natural habitat. The boxes consisted of a solid wooden frame covered with a wire screening of 5 mm. mesh, their dimensions being $30 \times 30 \times 20$ cm. (Fig. 1). The soil used in the boxes was obtained from the environments under consideration.

Groups of 45 clams were planted in each box. Usually, these were measured at five times: (i) the beginning of the experiment, February 28 to March 4, 1931, (ii) June 18 to 22 for obtaining "spring" growth, (iii) August 16 to 20 for "summer" growth, (iv) October 12 to 16 for "fall" growth, and (v) December 2 to 5 for "late fall" growth. During 1932, records were taken for the corresponding "spring" and "summer" periods.

Specimens were selected for planting so that the range of length within each group (45) was as small as possible (seldom exceeding 3 mm.) with a view to reducing the variability. In several experiments, designed to compare the effect of environmental conditions on rate of growth, specimens of the same length (pairs) were planted in the areas under comparison; therefore, at the beginning of the experiment, the groups possessed the same mean lengths and other mathematical constants. The advantage of "pairing" is to reduce the variability between the groups in order that the mean difference, if any, may be interpreted as due to environmental factors and not to variability between the groups.*

In all mathematical calculations, values have been computed to at least three places beyond the decimal, although recorded only to the nearest first or second place, depending on accuracy demanded.

III. Growth in Nature

Four beaches were selected for the study of growth under natural conditions. These are located at St. Andrews, New Brunswick; Economy Point, Nova Scotia; Clam Cove, New Brunswick; and Sissiboo River, Nova Scotia.

Opinion concerning the value of periodic rings for estimating the age of bivalve molluscs has not been unanimous. Mead and Barnes (14) question the validity of periodic rings on the valves of *Mya arenaria* for age determination. A study of the Bay of Fundy specimens, the valves of which differ significantly from those of southern regions, has indicated that certain rings are formed annually and constitute a valid basis for age determination. This validity is not the same throughout life.

In clams over four years of age, it is difficult to discern with assurance the first ring and frequently this also applies to the second. To obtain values representing the increment of growth during the first two or three years, large numbers of specimens, one, two, and three years of age were measured, since in young specimens these rings are more easily recognized. Cognizance must be taken of the fact that whereas mean values, obtained by measuring rings of a heterogeneous population ranging in age from three to twelve

*Standard error has been used throughout.

years, represent average growth increments, the result of average climatic conditions, those obtained by measuring a group of the same age imply a constant climatic factor. For this reason, the data obtained in these two ways are not directly comparable. To ascertain the extent of variation that exists, mean length values were obtained for the third "annual ring" (a) from

TABLE I
CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF
ESTIMATED ANNUAL RINGS OF *Mya arenaria* L.
IN THE ST. ANDREWS REGION

No. of specimens	Ring no.	Mean length in mm.	Standard deviation	C.V.
185	1	21.6 \pm 0.4	5.68 \pm 0.29	26.3 \pm 1.4
178	2	32.0 \pm 0.1	0.83 \pm 0.04	2.6 \pm 0.1
173	3	42.0 \pm 0.1	1.59 \pm 0.08	3.8 \pm 0.2
150	4	51.7 \pm 0.2	2.60 \pm 0.15	5.0 \pm 0.3
108	5	59.4 \pm 0.4	3.80 \pm 0.26	6.4 \pm 0.4
100	6	65.3 \pm 0.3	2.99 \pm 0.21	4.6 \pm 0.3

a heterogeneous population varying in age, and (b) from a population all of which were in their fourth year of growth, namely, 1932. The length of the third ring was, in the case of each specimen, a result of the same climatic conditions, *i.e.*, those of 1931. No very significant difference was indicated, the variation not exceeding 1.5 mm.

In the beginning, some other difficulties may be experienced in recognizing the probable annual ring as distinct from certain less prominent rings formed throughout the year, and in some cases, from those due to mechanical agencies (*cf.* 14, p. 55). The consistency of the shell is such that dark rings are often

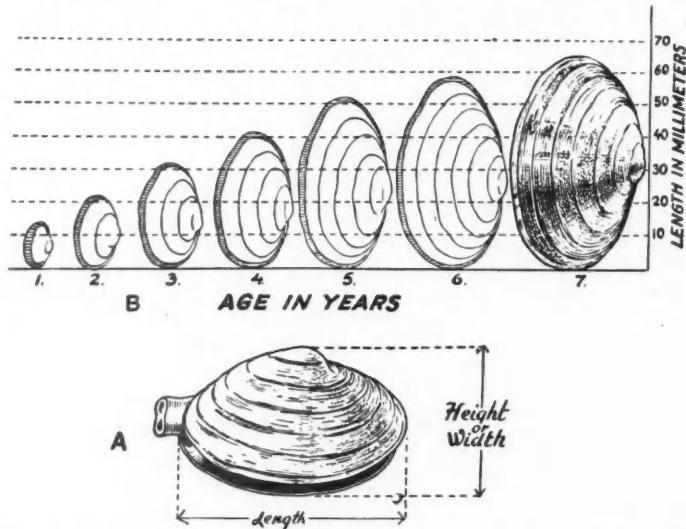


FIG. 2. A. Outline of *Mya arenaria* showing "annual rings" and the dimensions measured. B. Estimated lengths of *Mya arenaria* at different ages as shown by annual-ring measurements of the St. Andrews collection.

formed when the individual is disturbed. An example of this phenomenon is provided in Fig. 6, which shows the two faint lines produced when clams were taken up for measurement in June and August. Usually, however, the annual ring is wider, and more consistent and regular than the less prominent rings (Fig. 2).

On the basis of observations of specimens ranging in size from 13 mm. to 65 mm., grown in planting boxes, it appears that the mode of formation of disturbance and winter rings is quite similar (*cf.* 19). In the case of very old specimens* rings after the eighth or ninth are frequently indistinct, the annual increment of growth being small.

Collections were taken at approximately the same level on each beach, namely, eight feet above chart datum. For the purpose of comparing growth in nature at different levels on the same beach, an additional collection was made in Clam Cove at a level of sixteen feet.

1. ST. ANDREWS, N.B.

Specimens ranging in age from four to twelve years were collected for ring measurement from this beach in October, 1931. On account of the wearing away of the shell in the umbo region, a satisfactory value was not obtained for the length of the first ring. It is seen from Table II that the absolute yearly increment of length is quite similar for the third, fourth, and fifth years, namely, about 10 mm. The decrease during the sixth and seventh years is very marked (Figs. 2 and 3). The total lengths and widths of estimated annual rings have been plotted against age and are shown in Figs. 2 and 3.

TABLE II
YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* AS
INDICATED BY ESTIMATED ANNUAL-RING MEASUREMENTS IN THE ST. ANDREWS REGION

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage additions of length
185	1			
178	2	21.6		
173	3	32.0	10.3	47.8
150	4	42.0	10.1	31.5
108	5	51.7	9.7	23.0
100	6	59.4	7.6	14.8
	7	65.3	5.9	9.9

An expression of the yearly percentage additions of length, as shown in Table II, gives a more accurate idea of the decreasing growth increment with increasing age (Fig. 3). In this figure the initial length at the beginning of each year is given first, and immediately above is the absolute annual increment and then separately and higher up the corresponding percentage additions are represented.

*The largest clam examined by the writer in which the age was quite definitely indicated by the rings was in its seventeenth year, the total length being 98.4 mm. Starting with the second, the lengths of the respective rings are as follows: Number II—13.7 mm., III—28.3 mm., IV—47.5 mm., V—56 mm., VI—63.2 mm., VII—71.3 mm., VIII—78 mm., IX—82 mm., X—85.2 mm., XI—88 mm., XII—90.5 mm., XIII—92.8 mm., XIV—94.5 mm., XV—96.8 mm., XVI—98.4 mm.

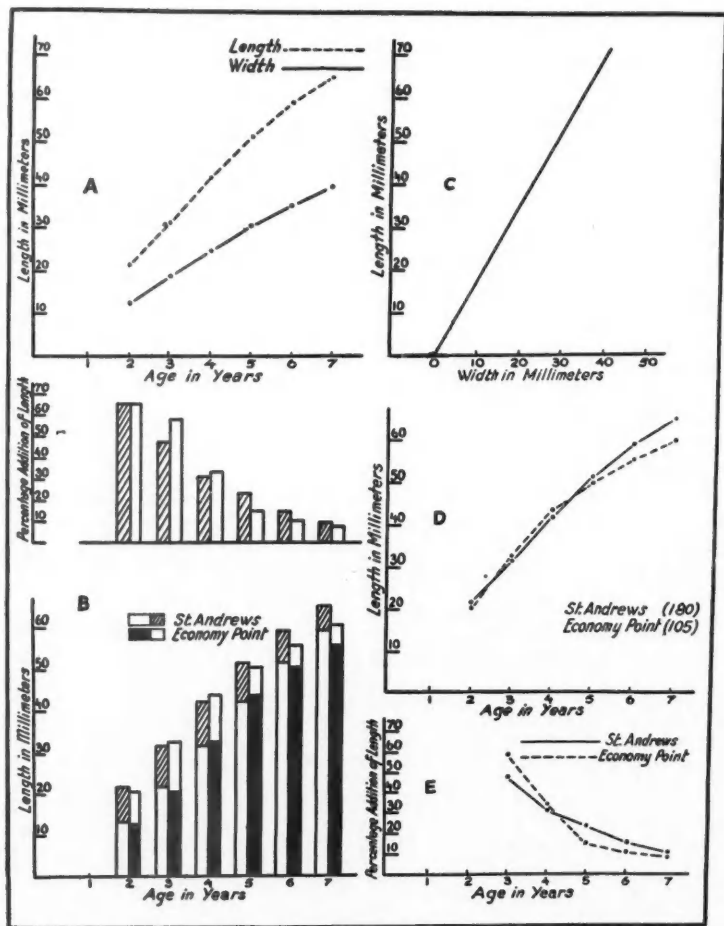


FIG. 3. A. Total lengths and widths of estimated annual rings plotted against age. This figure is based on mean values presented in Table I. B. Yearly increments of length of *Mya arenaria* as indicated by annual rings on valves. Length of first ring not definitely known. C. Length-width relation. D to E. Absolute yearly growth increments of *Mya* as shown by annual rings. D. Absolute growth; E. Percentage additions of length.

2. ECONOMY POINT, N.S.

The rate of growth of clams at the head of the Bay of Fundy is very little different from that of the mouth region as indicated by a study of the specimens collected at Economy Point (Tables III and IV). The mean lengths at the end of the second, third, fourth and fifth years are quite similar to the

lengths of the corresponding year rings of the St. Andrews collection (Table I). The yearly percentage additions in length for both collections of clams are given in graphic form (Fig. 3).

3. CLAM COVE, N.B.

Specimens were collected from two levels of the beach at Clam Cove in August, 1932, for the purpose of comparing rate of growth in nature as indicated by "annual ring" measurements with that in experimental planting boxes (see page 111). The collections were made at levels of 8 ft. and 16 ft. above chart datum.

It was not possible to secure a reliable mean value for the first ring from the specimens of either collection on account of the shell being worn away in the umbo region. The value, 3.9 mm., for the low level was obtained by measuring the single ring on specimens collected in May, 1932, and representing 1931 set. The figures in Table V showing the amount of growth during the first summer (1931) are not directly comparable with the remaining data and are included only for the reader's convenience.*

The average mean lengths of the rings of clams collected at the 16-ft. level are significantly less than those of the specimens from the low level (Table V). At the upper level, it is apparent that the absolute yearly increments of growth are approximately 50% of the corresponding increments for the less exposed level. At the eight-foot level the percentage addition in length during the third year is 87.0 as compared with 72.7 for the high level. The percentage additions for the succeeding years decrease markedly at both levels and, it is noted, in a fairly similar fashion (Table V).

*In the text, values for ring number one are, for convenience, referred to as though growth over a period of one year was implied. In reality, the period is less than four months, since it is not probable that set occurs in any abundance before August 1.

TABLE III
CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF
ESTIMATED ANNUAL RINGS OF *Mya arenaria* L.
IN THE ECONOMY POINT REGION

No. of specimens	Ring no.	Mean length in mm.	Standard deviation	C.V.
	1			
105	2	20.7±0.2	1.89±0.13	9.1±0.6
103	3	32.8±0.3	3.64±0.25	11.1±0.8
105	4	43.8±0.4	3.90±0.26	8.9±0.6
100	5	50.5±0.4	3.99±0.28	7.9±0.6
99	6	55.9±0.5	5.41±0.38	9.7±0.6
61	7	60.1±0.6	4.57±0.41	7.6±0.6

TABLE IV
YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* AS
INDICATED BY ESTIMATED ANNUAL-RING MEASUREMENTS
IN THE ECONOMY POINT REGION

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage addition of length
	1			
105	2	20.7		
103	3	32.8	12.1	58.4
105	4	43.8	11.0	33.7
100	5	50.5	6.7	15.2
99	6	55.9	5.4	10.7
61	7	60.1	4.2	7.5

The apparent 50% reduction in rate of growth at the more exposed zone of the beach is shown by a comparison of the growth rates produced at the two levels by clams possessing similar initial lengths at both levels, namely, 15.8 mm. and 28.1 mm. at the upper, and 15.7 mm. and 29.3 mm. at the lower level. Cognizance must be taken of the difference in age when making a comparison. These results are of particular interest on account of the similar retardation accompanying increase of exposure illustrated by field experiments (page 118). Thus, at the upper level, it may require nearly six years for a clam to reach a size ordinarily attained in three years at the lower and less exposed areas.

A comparison of the corresponding mean values obtained in 1931 for clams growing in the immediate vicinity of St. Andrews (Table I), with the Clam Cove results (1932) reveals a very close correspondence. With the exception of the value for the second ring, the difference usually does not exceed 2 mm.

The value representing the mean length at the end of the second year in the Clam Cove area is 15.7 mm. whereas that for the St. Andrews region is 21.6 mm. In order to check the accuracy of the former value, a separate group of one hundred clams, all of which were 1929 set, were measured, and the value thus obtained for the second ring was 16.4 mm., and for the third, 30.4 mm. These results confirm those for the second and third rings presented in Table V and imply that the difference is significant.*

TABLE V

YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* AS INDICATED BY ESTIMATED ANNUAL-RING MEASUREMENTS. (CLAM COVE REGION)

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage additions of length
<i>Level: 16 feet above chart datum</i>				
	1			
172	2	9.1		
152	3	15.8	6.6	72.7
125	4	22.5	6.7	42.3
49	5	28.1	5.6	25.1
28	6	32.7	4.5	16.2
16	7	36.1	3.4	10.5
<i>Level: 8 feet above chart datum</i>				
70	1	3.9		
242	2	15.7	11.8	301.8
245	3	29.3	13.6	87.0
148	4	40.5	11.2	38.3
67	5	48.5	8.0	19.8
57	6	57.5	8.9	18.4
39	7	63.8	6.3	11.0
22	8	69.4	5.6	8.8

*A similar result has been obtained by Fraser (6) at Dingle Beach on Mersey Estuary, near Liverpool, England, who states that specimens apparently showing two summers' growth average 1.7 cm. in February. (Level about 20 ft., chart datum being 4 ft.)

4. SISSIBOO RIVER, N.S.

Specimens for "annual ring" measurements were collected at an 8-ft. level on the Sissiboo beach in July, 1932. The mean values obtained correspond quite closely to those of the above-mentioned regions (Table VI). The data for all rings except number one were obtained from a single collection composed of clams of various ages, therefore the results are not likely to be appreciably influenced by yearly variations in climatic conditions. The value for ring number one, 4.9 mm., represents the average amount of growth in the case of clams which spawned in 1931, and was obtained by measuring a group of one hundred clams collected on July 14, 1932. In addition to measurement of the single ring present on specimens of this collection, records of total length were also made, the mean value being 14.5 mm. This represents an absolute increment of 9.6 mm. (195.9%) during approximately the first half of the growing season of 1932. The absolute increment obtained for the second year of growth is 15.4 mm., hence considerably more than one-half (9.6 mm.) of the growth during the second year had occurred prior to July 14. These results, signifying a relatively rapid growth rate in May and June, are quite similar to those obtained in the Passamaquoddy Bay region during 1931.

TABLE VI
YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* as
INDICATED BY ESTIMATED ANNUAL-RING MEASUREMENTS
IN THE SISSIBOO AREA
(Level; 8 feet above chart datum)

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage addition of length
100	1	4.9		
120	2	20.2	15.4	315.8
84	3	31.0	10.8	53.4
28	4	40.3	9.2	29.8
19	5	48.7	8.4	20.8
11	6	55.2	6.5	13.4

The percentage addition of length during the second year was very large, namely, 315.8; for the third year it was 53.4 and during succeeding years it gradually decreased until in the sixth year it was only 13.4. The mean value for the second ring, 20.2 mm., is significantly larger than that for the Clam Cove collection, whereas the mean length of the third ring is essentially the same in both groups (Tables V and VI). To check the accuracy of the lengths of the second and third rings of the Sissiboo collection, a separate group obtained from a similar level, all 80 specimens of which represented the 1929 set, was measured, and the mean length values calculated. The results obtained are as follows:—ring No. 2, 19.7 mm.; No. 3, 29.7 mm. The small differences obtained, of slightly over one mm., are obviously not significant, and constitute a reliable check of the accuracy of the values for the second and third rings. The absolute increments for the third, fourth and fifth years are essentially the same, namely, about 9.0 mm. and the corresponding increment for the sixth year is 6.5 mm.

To ascertain the differential growth rates occurring in very small clams growing under natural conditions, two groups, each of which contained thirty specimens in their second year of growth (1932), were selected by the method of random sampling. In one group the length of the single annual ring present ranged from 3.0 to 3.9 mm.; in the remaining group the corresponding range was 6.0 to 6.9 mm. The respective mean values obtained are 3.4 mm. and 6.2 mm.; these values will hereafter be referred to as the initial lengths of the respective groups. By measuring the total lengths, it has been possible to determine the increment of growth this year (1932) up to the date of collection (July 14) for specimens possessing initial lengths of 3.4 mm. and 6.2 mm. The average absolute increment for the smaller group was 9.0 mm. (264.7%). These values for the larger group were 9.8 mm. and 158.1%. It is shown that a marked decrease in the percentage increment occurs with increase in size, which fact has been previously suggested in Tables V and VI.

Table VII has been prepared by averaging the results of ring measure-

TABLE VII
AVERAGE YEARLY INCREMENTS OF GROWTH OF *Mya*
arenaria IN THE BAY OF FUNDY AS SHOWN BY
ANNUAL-RING MEASUREMENTS

Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage addition of length
1	4.4		
2	19.5	15.2	346.3
3	31.3	11.7	59.9
4	41.7	10.4	33.3
5	49.8	8.2	19.6
6	56.9	7.1	14.3
7	63.0	6.1	10.7

ments of the above-mentioned four collections (St. Andrews, Economy Point, Clam Cove and Sissiboo River). In general, the agreement of the results obtained from the various collections is sufficient to indicate that the averages presented give a fairly accurate picture of the growth phenomena characteristic of the Bay of Fundy Region (*cf.* 29, 30 and 31).

IV. Growth Experiments Under Natural Conditions

Attention has been given, firstly, to certain more important factors operating at the Clam Cove beach under what may for convenience be termed *normal conditions*, together with the growth rate characterizing these conditions; and secondly, to those factors which retard the growth processes as revealed by growth experiments set up in environments suitable for factorial analysis. The selection of these environments is beset with problems. Notwithstanding this fact, it is felt that certain positive evidence has accrued.

1. NORMAL CONDITIONS

Primary factors of the environment which are considered significant in the light of their effect on growth are period of submergence, circulation of water, food, salinity, temperature and soil. The beach at Clam Cove was studied for the purpose of evaluating certain of the above factors from the standpoint of growth.

(a) *Description of a Typical Beach—Clam Cove, N.B.*

The beach at Clam Cove is located on the east side of Deer Island near Fairhaven, N.B. The contour of the shore serves as a natural protection against heavy north and west winds as well as the excessive tidal action so strong in the nearby strait.

Size and slope. The area of this beach is approximately 55 acres, of which at least 30 acres are suitable for commercial digging. The slope is very gradual and quite uniform, there being a progressive diminution in the direction of the water. An idea concerning the degree of slope may be given by the fact that the distance between mean high-water and mean low-water level is about 400 metres and the average width nearly 200 metres.

Soil. The soil consists of a mixture of sand and mud, the proportions varying at different vertical levels. Above and near the high tide level sand predominates, and there are indications of some shifting. At a distance of about 50 metres below the mean high-water level, a high percentage of mud is present, the proportion increasing steadily at the lower levels. Below the mean low-water level there is a thin surface layer of soft mud which exerts a deleterious effect on growth. The soil is everywhere deep and in general unaffected by decomposing materials. At the lower levels, shallow tide-pools are present and an occasional seepage stream may be observed. Except at the very low levels, it is evident that the edaphic features of the environment are very favorable for the growth of *Mya arenaria*.

Salinity. The salinity records, although irregularly taken, indicate that the variation in salt content of the water at the head and at the mouth of the cove is slight at flood tide and that salinity changes take place in the soil water during exposure. In a paper on the physical factors of the sand beach (15) it is shown that the changes of salinity of soil water, caused by evaporation and subsurface seepage during exposure, are very slight. A change of 0.2% may occur in a large shallow tide-pool exposed for several hours.

Food. The excessive mixing of water in the Passamaquoddy region may be considered favorable for the production of food in this area. Unfortunately during the first two years of the investigation (1930-1931) the author was unable to obtain concrete data pertaining to the food of *Mya*. Indirect information on the food (diatoms) during the above period may be obtained from the studies of Davidson (3) regarding the abundance throughout 1931. These studies indicate that the diatoms begin to increase in March, reach their maximum in May or June, and then decline with, in some years, a less rapid decline in the autumn, usually in September or October. In Fig. 8 there is shown a logarithmic curve of diatom abundance during the period of the growth experiment.* It is of immediate interest to note that the diatom abundance during the fall and late fall periods of 1931 was very small, being significantly less than that of the previous year. Doctor Davidson states that the figures for August, 1931, are principally due to the dominance of *Chaetoceros socialis* which started on August 5 and continued until September.

*Personal communication from Dr. V. M. Davidson.

In 1930, *Chaetoceros socialis* (4–15 μ) was also abundant from August 20 to September 15, although at this time a larger-celled species, *Chaetoceros debilis*, (12–29 μ) was also present.

During the summer of 1932, an attempt was made to determine directly the more common organisms which compose the food of *Mya*. On account of the small size of the stomach and the difficulty of isolating all of the fluid contents, it has not been possible to secure exact quantitative data. The results of plankton tows of August 9, 1932, are given in Table VIII. Other samples taken in June, July, and August, which have been analyzed, present a similar picture, hence these data will be used as a basis for the present discussion. As shown in Table VIII, characteristic surface diatoms do not

TABLE VIII

PLANKTON ORGANISMS FOUND IN DIGESTIVE TRACTS OF *Mya* AND IN WATERS OVER BED FROM WHICH THE CLAMS WERE LATER EXAMINED
(St. Andrews, N.B., August 9, 1932)

Name	Some forms present in		
	Surface tows	Bottom tows	Stomach contents
<i>Chaetoceros</i> (3 species)	very common	common	
<i>Ceratulina</i>	common	rare	
<i>Rhizosolenia semispinosus</i>	common		
<i>Rhizosolenia styliformis</i>	rare		
<i>Pleurosigma</i>		very common	common
<i>Paralia sulcata</i>		common	very common
<i>Navicula</i>		occasional	common
<i>Nitzschia closterium</i>			rare
<i>Dinophysis</i> (with cell contents)			rare
<i>Tintinnus subulatus</i>	common	common	common
<i>Tintinnopsis campanula</i>	common	occasional	occasional
<i>Tintinnopsis cylindrica</i>	rare		occasional
<i>Peridinium crossipes</i>		occasional	very rare

occur in appreciable numbers in the stomach contents of this species. Certain tintinnids such as *Tintinnus subulatus* and *Tintinnopsis campanula* occur in both surface and bottom water layers. Dinoflagellates were found to be relatively scarce at the surface.

A study of the results obtained from bottom tows over the clam beds has revealed the presence of several species which are also abundant in the surface waters. In addition, *Pleurosigma* sp. and *Paralia sulcata* are quite abundant. These last-mentioned forms constitute the most numerous diatoms present in the stomach contents that were examined. The sample given in Table VIII perhaps lacks the usual variety of occasionals. From another sample collected on August 12 and dominated by *Paralia* and *Pleurosigma*, the following forms are recorded as very rare; *Chaetoceros compressus*, *Coscinodiscus* sp. *Nitzschia*, *Closterium*, *Navicula* sp. (sometimes very abundant) and *Rhizosolenia semispinosa*.

Among the tintinnids of the stomach contents *Tintinnus* (*Helicosthomella*) *subulatus* is probably the most abundant species. Frequently the shell contents were absent, which might indicate that they had been digested. However, inasmuch as the clams were either preserved or examined immediately after collection, and also since the diatoms were usually in a well preserved condition, it is believed that many forms of this genus are without cell contents when taken in by the clam.

Dr. Braarud* states that he frequently finds empty tintinnids in plankton collections from this region, which fact supports our contention. Dinoflagellates are represented chiefly by *Dinophysis* sp., often very abundant, and a species of *Peridinium* listed as very rare. As in the case of the tintinnids, specimens of *Dinophysis* sp. are usually without cell contents. The silico-flagellate *Distephanus speculum* has also been observed.

It is seen that diatoms taken in by *Mya arenaria* are bottom forms which are also frequently present in the bottom plankton tows (*Paralia*, *Pleurosigma* and *Navicula*) and neritic species. Although tintinnids, the most abundant zooplanktons recorded from stomach contents, are commonly present, their numbers are often due to empty shells which were empty when taken in. The data, although scanty in some respects, are indicative of the major role played by diatoms in the food of *Mya arenaria*, during the period studied.

Table IX shows the approximate age of clams belonging to the various length classes at two different levels on the beach at Clam Cove. These age determinations are based on annual-ring measurements of 1932 (Table V). It may be pointed out that whereas a clam at the lower levels is able to reach a marketable size (40 mm. in length) in three or four years, approximately double this period is required at the upper levels.

TABLE IX
APPROXIMATE AGE OF CLAMS BELONGING TO THE
SEVERAL LENGTH CLASSES STUDIED IN
THE CLAM COVE REGION

Length class, mm.	Approximate age in years at	
	7-9 ft. level	16 ft. level
5 - 14	1 - 2	1 - 2+
15 - 24	2 - 3	3 - 4
25 - 34	3 - 3+	5 - 6
35 - 44	3+ - 4+	7 - 8
45 - 54	4+ - 5+	8 - 10
55 - 64	5+ - 7	probably over 10
65 - 74	7 - 9	

Biotic relations. Several species of Polychaete worms such as *Nereis virens* (about 24 per square metre) and a Nemertine (*Cerebratulus* sp.) in small numbers were recorded. A few small communities of the sea mussel *Mytilus edulis* occur but not in sufficient numbers to limit the *Mya* population (cf. 17).

Little plant life is present on this flat. The surface of the mud is in places covered with diatoms, including *Lichmorpha*, *Navicula* and *Nitzschia*. Occasional tufts of *Fucus vesiculosus* occur, and low down near the mean low tide level in the larger tide-pools some *Zostera marina* is present (18).

*Assistant Expert for the phytoplankton investigations of the International Passamaquoddy Fisheries Commission.

(b) Extent of Growth

The annual extent of growth has been obtained by summing increments for the five periods which are designated "spring" (111 days), "summer"

(59 days), "fall" (57 days) and "late fall" (50 days).

Several experiments were continued in 1932 to compare the growth rate during 1931 with that of the corresponding periods of the following year. Measurements made during the period December 3 to 8, 1931, indicate that no appreciable growth took place during the "late fall" period, hence it is believed that during the period from the middle of October to March 1, no significant growth occurred.

TABLE X
TEMPERATURE, IN DEGREES CENTIGRADE, TAKEN AT THE
BIOLOGICAL STATION WHARF, ST. ANDREWS,
DURING 1931 AND 1932

Months	1931		1932	
	Mean daily temperature		Mean daily temperature	
	Water	Air	Water	Air
January	2.96	-6.42	3.01	-1.98
February	1.71	-5.52	0.71	-7.97
March	1.68	0.72	0.72	-2.91
April	3.72	5.34	3.29	3.34
May	6.63	10.26	6.29	9.57
June	9.63	14.85	9.03	14.46
July	12.37	18.22	11.37	16.46
August	13.23	18.18	13.11	17.58
September	12.23	13.43	12.91	16.04
October	10.93	9.52	11.35	9.12
November	8.58	5.65		
December	4.71	-3.37		

TABLE XI
CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF SMALL AND LARGE CLAMS USED
IN EXPERIMENTS OF 1931

Box	Location	Level, ft.	No. of specimens	Mean length during experiments		Standard deviation	C. V.
				At beginning	At end		
A	Clam Cove	18	45	18.4 ± 0.1		1.04 ± 0.11	5.7 ± 0.6
			45		23.9 ± 0.2	1.31 ± 0.13	5.4 ± 0.5
B	Clam Cove	7	45	18.5 ± 0.1		0.96 ± 0.10	5.2 ± 0.5
			44		30.5 ± 0.4	2.45 ± 0.26	8.1 ± 0.8
C	Clam Cove	5	45	18.4 ± 0.1		0.94 ± 0.09	5.1 ± 0.5
			41		28.6 ± 0.4	2.67 ± 0.29	9.3 ± 1.0
A1	Clam Cove	18	45	26.4 ± 0.1		0.84 ± 0.08	3.2 ± 0.3
			44		31.7 ± 0.2	1.60 ± 0.17	5.0 ± 0.5
B1	Clam Cove	7	45	26.6 ± 0.1		0.97 ± 0.10	3.7 ± 0.4
			42		34.7 ± 0.4	2.77 ± 0.30	8.1 ± 0.9
C1	Clam Cove	5	45	26.5 ± 0.1		0.77 ± 0.08	2.9 ± 0.3
			42		35.2 ± 0.4	2.44 ± 0.26	6.9 ± 0.8
D	St. Andrews	5	45	19.3 ± 0.2		1.24 ± 0.13	6.4 ± 0.6
			44		32.0 ± 0.1	0.71 ± 0.07	2.2 ± 0.2
D1	St. Andrews	13	45	18.3 ± 0.1		0.92 ± 0.09	5.0 ± 0.5
			41		26.3 ± 0.3	1.85 ± 0.20	7.0 ± 0.8
E	St. Andrews	7	45	22.2 ± 0.1		0.89 ± 0.09	4.0 ± 0.4
			45		32.0 ± 0.4	2.82 ± 0.29	8.8 ± 1.0
F	Waweig Creek	13	45	22.3 ± 0.1		0.90 ± 0.09	4.0 ± 0.4
			38		24.3 ± 0.2	1.01 ± 0.11	4.2 ± 0.5

(1) Annual growth

Annual growth records have been obtained by summing the increments of growth for each season. Consequently, they are likely to be somewhat less than the true values on account of the probable retardation in rate of growth caused by the clams being disturbed for the purpose of measurement.

The annual growth increments of small (initial length, 18.4 mm.) and large specimens (initial length, 26.5 mm.) are treated separately. For small clams, the maximum absolute increment of growth obtained was 12.8 mm. (66.2%) for specimens which possessed an initial length of 19.3 mm. (Table XII).

Results obtained at the beaches near St. Andrews and at Clam Cove indicate the influence of beach level on the rate of growth of small clams. Fig. 4, C shows the growth increments for specimens of approximately the

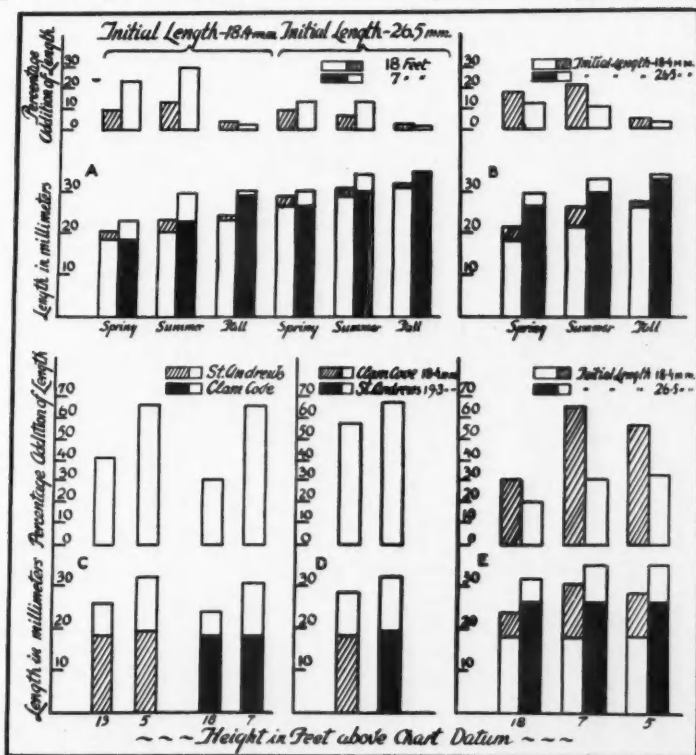


FIG. 4. A. Seasonal growth of "small" and "large" clams at two levels on beach at Clam Cove, N.B. B. Comparison of seasonal growth of small and large clams expressed as absolute increment of length and as percentage addition of length. Figure is based on average records for three levels on the beach at Clam Cove, N.B. C. Comparison of annual growth expressed as absolute increment of length and percentage addition of length at different levels on beaches at St. Andrews and Clam Cove, N.B. D. Comparison of annual growth of clams of similar initial lengths at the same level (five feet above chart datum) on two beaches located at Clam Cove and St. Andrews, N.B. E. Comparison of the extent of growth of small and large clams at three levels of the beach at Clam Cove.

TABLE XII
INCREMENTS OF SEASONAL GROWTH OF SMALL AND LARGE CLAMS USED IN EXPERIMENTS OF 1931

Box	Location	Level, ft.	Initial mean length, mm.	Amount of spring growth, mm.	Addition of length during spring, %	Amount of summer growth, mm.	Addition of length during summer, %	Amount of fall growth, mm.	Addition of length during fall, %	Total growth, mm.	Addition of length during entire period, %
A	Clam C.	18	18.4	1.8	9.7	2.7	13.4	1.0	4.5	5.5	30.0
B	Clam C.	7	18.5	4.3	23.5	6.9	30.0	0.9	3.0	12.1	65.3
C	Clam C.	5	18.4	3.8	20.9	4.6	20.8	1.9	7.2	10.4	56.5
A1	Clam C.	18	26.4	2.6	9.6	1.9	6.6	0.8	2.7	5.3	20.0
B1	Clam C.	7	26.6	3.7	13.7	4.1	13.5	0.4	1.3	8.2	30.7
C1	Clam C.	5	26.5	4.1	15.4	3.2	10.4	1.6	4.9	8.9	33.5
D	St. And.	5	19.3	5.9	30.5	4.7	18.8	2.1	7.2	12.8	66.2
D1	St. And.	13	18.3	3.0	16.3	3.4	15.6	1.3	5.1	7.7	41.8
E	St. And.	7	22.2	3.5	15.7	3.7	14.5	2.6	8.9	9.8	44.4
F	Waveig C	13	22.3	1.6	7.4	0.4	1.6	0.0	0.0	2.0	9.2

same initial length planted at different levels (5 and 13 ft. above chart datum) on beaches located in the St. Andrews vicinity. The percentage addition of length at the upper level was 35% less than that obtained at the five-foot level.

The annual absolute increment of growth in Box B (Clam Cove) at a level of seven feet above chart datum was 12.1 mm. (65.3%). A comparison of these results with those obtained for specimens of similar initial lengths planted in the same beach but at a different level (18 ft.) reveals a reduction of over 50% in absolute as well as percentage increments (Table XII and Fig. 4, C). The total absolute increment during 1931 and 1932 (until the end of the summer period) at the eighteen-foot level was 9.7 mm. (52.8%) in comparison with 19.0 mm. (102.9%) at the seven-foot level (Table XIII). A confirmation of this reduction in growth rate accompanying decrease in level has been demonstrated by the results obtained from annual-ring measurements of growth in nature (page 104).

Data obtained from experiments in Boxes C and D have indicated that under comparable conditions the rates of growth on two different beaches near the Biological Station are of a similar order of magnitude.

The maximum annual growth of specimens 26.5 mm. long (large clams) was 8.9 mm. (33.5%) and took place at a level of five feet on the beach at Clam Cove—Box C 1 (Table XII).

The influence of exposure on the rate of growth is shown by comparing the growth in Box C 1, 8.9 mm., with that in Box A 1, 5.3 mm., located 18 ft. above chart datum (Table XII). Again, as in the case of the small clams, a reduction in rate of growth accompanying increase in level is evident, but here the decrease is not so conspicuous. The reduction in percentage addition of length is about 30% as compared with 50% for the small clams.

The experiments, which were continued throughout the spring and summer periods of 1932, showed the total absolute increment of growth to be 8.5 mm. (32.3%) at the higher level, whereas at the seven-foot level (Box B) the increments were 14.5 mm. and 55.0% respectively (Table XIII).

Growth increments of small and large clams planted at the same and different beach levels indicate a comparatively slow rate of growth of the large clams (26.5 mm.). The annual growth increments for small and large clams at three levels are given in Table XII and Fig. 4, E. The differences observed in those growing at levels of five and seven feet are not large, the increment of growth in Box D (level, five feet) being 1.6 mm. less than that in Box B. This is due to excess of surface silt at the lower level. (See page 122).

In general, it may be said that the rate of growth of *Mya arenaria* in the Bay of Fundy region is small and the growing period of short duration.

(2) Seasonal growth

A study of the trend of growth throughout each season is of interest from the viewpoint of the fluctuation in environmental conditions, especially temperature and food. The growth rates of small and large clams are presented separately.

TABLE XIII
AMOUNT OF SEASONAL GROWTH DURING 1931 AND 1932 AT CLAM COVE, N.B.

Seasons	Average length at end of each season, mm.	Seasonal absolute increment of growth, mm.	Seasonal addition of length, %	Average length at end of each season, mm.	Seasonal absolute increment of growth, mm.	Seasonal addition of length, %
Initial length 18.4 mm. (Year 1931)						
Box A—Level 18 ft.			Box B—Level 7 ft.			
Spring	20.1	1.8	9.7	22.8	4.3	23.5
Summer	22.9	2.7	13.4	29.7	6.9	30.0
Fall	23.9	1.0	4.5	30.5	0.9	2.9
Total		5.5	30.0		12.1	65.3
(Year 1932)						
Spring	26.9	2.9	11.9	34.1	4.0	13.5
Summer	28.2	1.3	4.9	37.1	2.9	8.6
Total for 1932		4.2	17.4		6.9	23.0
Total for 1931 and 1932		9.7	52.8		19.0	102.9
Initial length 26.4 mm. (Year 1931)						
Box A1—Level 18 ft.			Box B1—Level 7 ft.			
Spring	28.9	2.5	9.6	30.2	3.7	13.7
Summer	30.9	1.9	6.6	34.3	4.1	13.5
Fall	31.7	0.8	2.7	34.8	0.4	1.3
Total		5.3	20.0		8.2	30.7
(Year 1932)						
Spring	33.9	2.2	6.9	37.6	3.2	9.3
Summer	34.9	1.0	3.0	40.7	3.1	8.4
Total for 1932		3.2	10.1		6.4	18.5
Total for 1931 and 1932		8.5	32.3		14.5	55.0

The seasonal distribution of growth of small clams under favorable conditions, is shown by the results obtained in Box B (Tables XII and XIII and Fig. 4, A). The maximum seasonal growth occurs in summer, spring growth occupying second place; the difference, in this instance, being 2.6 mm. (6.5%). The fall growth is very small, being less than 1 mm. (3.0%) as compared with 6.9 mm. during the summer season. This phenomenon has been found to be of quite general occurrence in the Passamaquoddy region.

In Table XII and Fig. 4, A the relative increments of seasonal growth at different levels are shown for both small and large specimens. Considering at first the small clams, the greatest growth occurring at levels of 7 ft. and 18 ft.

is during the spring and summer seasons. In the fall, the largest percentage addition of length, 7.2, was obtained at the five-foot level. To throw more light on the relative seasonal variations in growth rate for this region, Fig. 4, B, based on average records for the three levels, 5, 7 and 18 ft., is presented. The figure shows the average seasonal absolute increments as well as the average percentage additions in length. The extent of growth during the spring and summer seasons is practically identical (addition of length about 20%), whereas, in the fall it is reduced to less than 5%.

Fig. 5, C shows the seasonal growth obtained in small clams of similar initial lengths (18.3-19.3 mm.) on nearby beaches. In Box D1 planted at the 18-foot level, spring and summer increments are quite similar, 3.0 mm. and 3.4 mm. respectively, whereas during the fall period the length increment is only 1.3 mm. At the lower level the growth rate is greater during all seasons, especially spring.

The Clam Cove experiments with both the small and large specimens at levels of 18 and 7 ft. above chart datum were continued during the spring and summer periods of 1932. The total amount of growth during these seasons in the case of small specimens at the 18-foot level was 4.2 mm. (17.4%) and at the lower level, the absolute increment was 6.9 mm. (23.0%). These results indicate a somewhat slower rate than during the same period of 1931 (Table XIII).

Comparing the spring increments obtained during 1931 and 1932, little difference is observed whereas during the summer period the rate was somewhat less during 1932 (Table XIII). In order to compare the summer increments during 1931 and 1932, a box containing 45 clams possessing a mean length of 30.1 mm. was planted at a level of seven feet above chart datum at Clam Cove. The absolute increment obtained for the summer period was 3.0 mm. (9.9%). The values obtained in a comparable experiment during the same period of 1931 were 4.1 mm. and 13.5% respectively (Table XIV). These results suggest that the growing conditions in Clam Cove during the summer of 1932 were slightly less favorable for *Mya* than in the corresponding period of the previous year. Other data presented in another part of this paper corroborate these results. A comparison of the spring and summer increments during 1932 for specimens possessing similar initial lengths, namely, 34.4 and 34.1 mm., shows a slight retardation during the summer season (Table XIV).

A comparison of the seasonal amount of growth of small clams, 18.4 mm., taken from the same level on different beaches is presented in Table XII and Fig. 5, A. Comparing the percentage additions of length it is seen that, with the exception of the spring season, results are almost identical.

An examination of the results of the seasonal growth rate of large clams, 26.5 mm., indicates a growth of 4.1 mm. (Box C 1) in the spring at a level of five feet. This is the maximum absolute increment of length obtained for any single season. The smallest amount of growth for this length class occurred in the fall season when the absolute increment was 1.6 mm. (Table

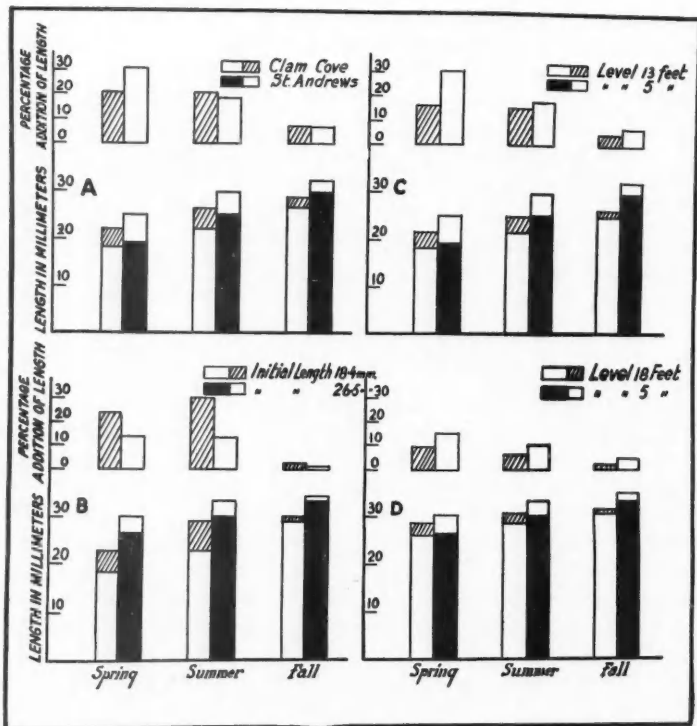


FIG. 5. A. Comparison of seasonal growth of clams of similar initial lengths at the same level on beaches at St. Andrews and Clam Cove, N.B. B. Comparison of seasonal growth of "small" and "large" clams at a level of seven feet above chart datum on beach at Clam Cove, N.B. C. to D. Comparison of seasonal growth of clams of similar initial lengths at two different levels on beach at St. Andrews (C) and Clam Cove (D).

XII and Fig. 4, A). As is shown in Fig. 4, B, the percentage of growth in spring is slightly in excess of the summer growth; however, both are far greater than the fall increment.

The rates of growth of large clams on different levels of the same beach, are indicated in Table XII and Figs. 4, A, 4, E and 5, D. As noted above, growth is less at the upper levels throughout each season. The greatest differences were obtained during the spring and summer seasons.

A comparison of the seasonal growth of small and large clams at the same level under favorable conditions is presented in Table XIII and Fig. 5, B. The spring and summer increments are approximately the same in the large specimens, whereas the small clams evidenced a somewhat larger percentage addition of length (30.0%) during the summer season (Boxes B and B 1). A further comparison of growth rate throughout the three seasons in the small and large clams is presented in Fig. 4, B. This figure indicates that the

percentage additions of length of both sizes are of similar magnitude during spring and summer and that the growth of the small clams exceeds that of the larger specimens throughout each season.

TABLE XIV

COMPARISON OF THE EXTENT OF GROWTH OF *Mya arenaria* DURING THE SPRING AND SUMMER PERIODS OF 1931 AND 1932

Year	Spring			Summer			Total absolute increment of length, mm.	Total percentage addition in length
	Length at beginning of period, mm.	Absolute increment of length, mm.	Percentage addition in length	Length at beginning of period, mm.	Absolute increment of length, mm.	Percentage addition in length		
1931	26.4	2.5	9.6	28.1	1.9	6.6	4.5	16.9
1932	24.1	2.9	11.9	26.9	1.3	4.9	4.2	17.3
1932	34.4	3.2	9.3	34.1	2.9	8.6		
1931				30.2	4.1	13.5		
1932				30.2	3.0	9.9		

The 1931 experiments with large specimens at levels 7 and 18 feet at Clam Cove, continued during the spring and summer periods of 1932, indicate a general retardation in rate accompanying an increase of size. As a consequence, the differential rates for the two levels exhibit less variation and, moreover, this variation is further minimized by the lower initial lengths at the upper level tending to give a more rapid rate of growth which affects, to a marked degree, the retarding influence of exposure. The total absolute increment obtained for both periods of 1932 at the 18-foot level is 3.2 mm. (10.1%) as compared with 6.4 mm. (18.5%) at the 7-foot level. It is apparent that the retarding influence of exposure is not greatly affected by the difference in initial length of 3.7 mm. At the 18-foot level, the increments for the spring period were about double those for the summer season (Table XIII) whereas, at the lower level, the variation was much less pronounced.

For the purpose of comparing the spring growth in nature during 1932, at these levels, with that occurring in the experimental boxes, clams of various sizes were selected for measurement and grouped according to the ranges listed in Table XV. By recording the total length and the length of the 1931-1932 ring for each specimen, it was possible to calculate the mean spring increment for clams falling within the various ranges. The purpose of this experiment was to extend our knowledge of seasonal growth rate over a wider range of size and also to determine the difference, if any, between the amount of growth in nature and that in experimental boxes.

From Table XV (18-foot level) it is seen that the maximum percentage addition of length for the sizes considered occurs in those ranging from 8.0 to 11.9 mm., the mean length being 9.6 mm. For the larger sizes, there is a pronounced decline in percentage increments, the percentage addition for specimens having a mean initial length of 38.0 mm. being 2.6. Comparing

these results with those obtained by using planting boxes (Table XVI) it is shown that for an initial length of 29.0 mm. at the 18-foot level, the percentage increment was 6.9 whereas, in the planting boxes, the addition was 6.9% when the initial length was 31.4 mm. Again, comparing results obtained from Box A (initial length, 24.0 mm.) with the percentage increment in nature for specimens having an initial length of 25.5 mm., less agreement is found, the difference being 2.9%.

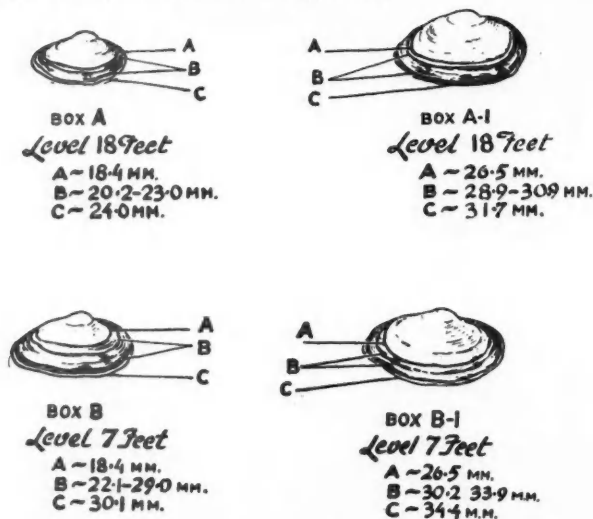


FIG. 6. *Mya arenaria*. Extent of growth of small and large sizes at two levels of beach at Clam Cove during experiment in 1931. A. Initial length. B. Length at end of "spring" and "summer" seasons. C. Length at end of experiment. Each figure, reduced about one-third, represents mean type of lots of 45 specimens.

Considering in a comparative way (Table XV) the data obtained at the lower level (7 feet), a gradual decline in percentage increment of growth accompanying increase in size is observed, the addition being 5.7% for clams possessing an initial mean length of 49.4 mm. In nature, clams possessing an initial length of 29.9 mm. show a percentage increment of 13.1 as compared with 13.3 for specimens having an initial length of 30.1 mm. planted in Box B (Table XVI). For the size used in Box B 1, a fairly good agreement has also been obtained.

Comparing the spring growth rate at levels 7 and 18 feet, a similar relationship is found to that indicated by annual-ring measurements for these levels (Table V), the rate of growth at the eighteen-foot level being reduced approximately 50%. The data presented in Table XV support the results of the planting experiments as well as the conclusion on differential growth rate at the two levels and, furthermore, broaden the picture of growth in nature during the spring season of 1932.

TABLE XV

EXTENT OF SPRING GROWTH DURING 1932 AT CLAM COVE, N.B. AS SHOWN BY
ANNUAL-RING MEASUREMENTS

No. of specimens	Range	Length at beginning of spring period, mm.	Amount of spring growth, mm.	Addition in length during spring period, %
Level; 18 feet above chart datum				
25	8.0 - 11.9	9.6	3.1	32.6
24	12.0 - 15.9	13.8	2.6	18.8
20	16.0 - 19.9	17.4	2.0	11.6
36	20.0 - 23.9	22.3	2.5	11.3
36	24.0 - 27.9	25.5	2.3	9.0
12	28.0 - 31.9	29.0	2.0	6.9
13	32.0 - 35.9	33.8	1.2	3.5
14	36.0 - 39.9	38.0	0.9	2.6
Level; 7 feet above chart datum				
40	24.0 - 27.9	26.4	4.0	15.2
50	28.0 - 31.9	29.9	3.9	13.1
36	32.0 - 35.9	33.6	2.6	7.9
30	36.0 - 39.9	37.5	2.7	7.3
17	40.0 - 43.9	41.9	2.8	6.7
17	44.0 - 47.9	45.5	2.7	5.9
10	48.0 - 51.9	49.4	2.8	5.7

On a previous page, reference has been made to the decline in growth rate with increase in size, the most marked retardation occurring during the early years (Tables II, IV and V). A similar phenomenon is evident from the results of spring growth presented in Table XV. In the case of clams having initial lengths of 9.6 mm. and 22.3 mm., the reduction of percentage increment was approximately two-thirds

(from 32.6 to 11.3%) for the spring season. In Table V it is seen that specimens possessing similar initial lengths also exhibit a reduction in percentage increment of about 60% during a one-year period. Other initial lengths are not such as to permit direct comparison. However, the general trend of diminishing increment is similar in both sets of data.

TABLE XVI

COMPARISON OF THE EXTENT OF GROWTH IN NATURE AS
SHOWN BY ANNUAL-RING MEASUREMENTS WITH THAT
IN PLANTING EXPERIMENTS AT CLAM COVE,
N.B. DURING SPRING PERIOD OF 1932

Growth in	Initial length, mm.	Absolute increment of growth, mm.	Percentage addition of length
Nature	29.0	2.0	6.9
Planting experiment	31.4	2.2	6.9
Nature	25.5	2.3	9.0
Planting experiment	24.1	2.9	11.9
Nature	29.9	3.9	13.1
Planting experiment	30.1	4.0	13.3
Nature	33.6	2.6	7.9
Planting experiment	34.4	3.2	9.3

The character of the decline in growth rate with increase in size for the 7-foot level is likewise comparable (Table V and XV). During the spring season of 1932, the percentage increment was 6.7 for clams having an initial length of 41.9 mm., *i.e.*, about 50% of the corresponding addition for specimens with an initial length of 29.9 mm. In Table V, the reduction for clams possessing similar initial lengths is shown to be 45.7% (from 38.3% to 19.8%) during one year.

These results are of particular value in strengthening our position with regard to the relative decline in growth accompanying increase in length.

In connection with seasonal growth rate, it is of interest to note the growth which occurred on the beach at Harbor de Lutre, Campobello Island, during the period from July 9 to September 24, 1931 (78 days) (Table XVII). Clams

TABLE XVII
CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF *Mya arenaria*
USED IN EXPERIMENTS AT CAMPOBELLO ISLAND, N.B. (LEVEL: 7 FEET ABOVE DATUM)

Time	No. of specimens	Mean length, mm.	Standard deviation	C. V.	Mean width, mm.	Standard deviation	C. V.
(Location—Harbor de Lutre)							
Planted July 9	45	26.3 ± 0.1	0.78 ± 0.08	2.9 ± 0.3	16.7 ± 0.1	0.69 ± 0.07	4.1 ± 0.4
Removed Sept. 24	43	30.5 ± 0.3	2.14 ± 0.22	7.0 ± 0.8	19.0 ± 0.2	1.10 ± 0.11	5.8 ± 0.4
(Location—Cranberry Point)							
Planted July 7	45	26.3 ± 0.1	0.78 ± 0.08	2.9 ± 0.3	16.6 ± 0.1	0.69 ± 0.07	4.2 ± 0.4
Removed Sept. 24	37	29.3 ± 0.3	2.07 ± 0.24	7.1 ± 0.8	18.5 ± 0.2	1.42 ± 0.16	7.7 ± 0.8

were planted at a low level on the flat and the average amount of growth per specimen was 4.2 mm. (15.9%). These results, although not directly comparable with the Clam Cove data on account of the difference in the time factor, nevertheless suggest that the growth rate is quite similar on the two beaches.

Some interesting results pertaining to seasonal growth were obtained during the fall of 1930 (15). Small specimens (initial mean length, 13.5 mm.) submerged continuously off the Biological Station float, exhibited a growth of about 5.6 mm. (41.4%) during the period August 8 to October 11, 1930 (43 days). Again, specimens (15 in number and 13.6 mm. in mean initial length) planted at a low level on a beach also near the Biological Station showed a similar growth, namely, 5.4 mm. (39.8%) during approximately the same period of time. The last mentioned group of clams was replanted in October and measured again on February 4, 1931. The average growth during this period was 2.7 mm. (percentage addition of length, 14.28). These

results are indicative of two main facts, first, that growth during the so-called fall season was, during 1930, significantly more than that of 1931, and second, that growth continued after the middle of October, probably until about December 1, during 1930 (Fig. 8). These results are significant in the light of the small growth after September 1, 1931, and will be discussed in a later part of this paper.

On the basis of the above presentation it is seen that a considerable variation exists with regard to seasonal growth of *Mya arenaria* in the Passamaquoddy region.

2. LIMITING CONDITIONS

The complexity of the factors composing a marine environment is such as to render difficult factorial analysis based on field experiments. The results obtained, however, present an expression of the amount of growth occurring in a variety of environments and help to evaluate the relative importance of certain factors in respect to their effect on rate of growth of this form.

(a) Description of areas where experimental boxes were planted.

To determine the influence of certain environmental factors (soil, salinity, sulphide, etc.) on the rate of growth of *Mya arenaria*, planting boxes were placed at various levels on the same and different beaches (Table XVIII).

(b) Extent of growth.

Three factors exerting a limiting effect on rate of growth have received attention.

(1) *Soil.* Two types of soil which exert a limiting influence on the growth of this species are considered; soft mud with surface layer of silt, and shifting sand. Two lots of clams ($n=45$, initial lengths 26.3 mm.) were planted at Cranberry Point and Harbor de Lutre, Campobello Island, under similar conditions as far as was possible to determine, except that at Cranberry Point the soil was softer and, over the surface, a thin layer of fine silt was very conspicuous (period of experiment, July 8 to September 24, 1931; 78 days). The amount of growth at Harbor de Lutre was 4.2 mm. (15.8%) as compared with 3.0 mm. (11.4%). The difference is probably due to the deleterious mechanical influence of silt on the rate of growth. Practically no clams are present at this level on the Cranberry Point flat, whereas a considerable number occur at Harbor de Lutre where soil conditions are favorable (Table XVII).

Again, a similar deleterious effect is evident in the case of growth in Box C planted at a low level (five feet) on the beach at Clam Cove (Table XII and Fig. 4, E). From an examination of these data, it is seen that with the exception of fall growth, seasonal as well as total difference in percentage additions of length is as high as 9. This decrease in growth rate is correlated with a permanent surface layer of silt, the importance of which, as a factor limiting growth in *Mya arenaria*, is mentioned elsewhere (p. 135).

Shifting sand is an important factor limiting the growth of *Mya arenaria* in the Bay of Fundy region. The almost total absence of a *Mya* population on a certain sand beach located near Joe's Point, St. Andrews, suggested the following experiment carried on during the period August 29 to October 12, 1931, *i.e.*, 44 days (16). When the clams (initial mean length, 14.1 mm.;

TABLE XVIII
DESCRIPTION OF BEACHES WHERE BOXES WERE PLANTED

Box	Beach	Level in feet	Soil type	Salinity per mille of tidal water
A and A1	Clam Cove	18	Fine sand and a little mud	About 31.0
B and B1	Clam Cove	7	Mud and sand ratio about 3 : 1	About 31.0
C and C1	Clam Cove	5	Mud and sand ratio about 8 : 1; considerable silt on surface	About 31.0
D	Near St. Andrews	5	Mud and sand ratio about 2 : 1	About 31.0
D1	Near St. Andrews	13	Mud and sand ratio about 2 : 1	About 31.0
E	Near St. Andrews	7	Mud and sand ratio about 5 : 1; high sulphide content	About 31.0
F	Waweig Creek	12	Mud and sand	0.8—26.0
G	Cranberry Point	7	Mud and sand ratio about 8 : 1; surface layer of silt	About 31.0
H	Harbor de Lutre	7	Mud and sand ratio about 3 : 1	About 31.0

$n=15$) were examined at the end of the period only four were alive. Examination of the specimens showed that two had been dead only a short time. All specimens were measured and the mean length value computed was 15.7 mm. The results show that the rate of growth is very slow in spite of the low level of about 10 feet and indicate that the average increment of growth was only about 1.6 mm. (11.4%). Furthermore, the deleterious effect of shifting sand on the survival of a clam population is demonstrated.

(2) *Salinity*. An experiment to determine the limiting effect of salinity on growth rate in nature was conducted on the intertidal zone of the Waweig Creek at a level of 13 feet above chart datum (Box F) (Table XII and Fig. 7, A). Since it is located several miles from the tidal waters on Passamaquoddy Bay, the range of salinity is very great—from about 30 o/oo (high tide) to about 2 o/oo (low tide), when exposed to ebbing water practically fresh. The chief growth occurred during the spring season, the absolute increment being 1.6 mm. (7.4%) as compared with a total for all seasons of 2.0 mm. (9.2%). The latter figure is less than one quarter of that which occurs under normal conditions at Joe's Point (Box D 1). The seasonal distribution of growth in comparison with that of Joe's Point is shown in Fig. 7, A.

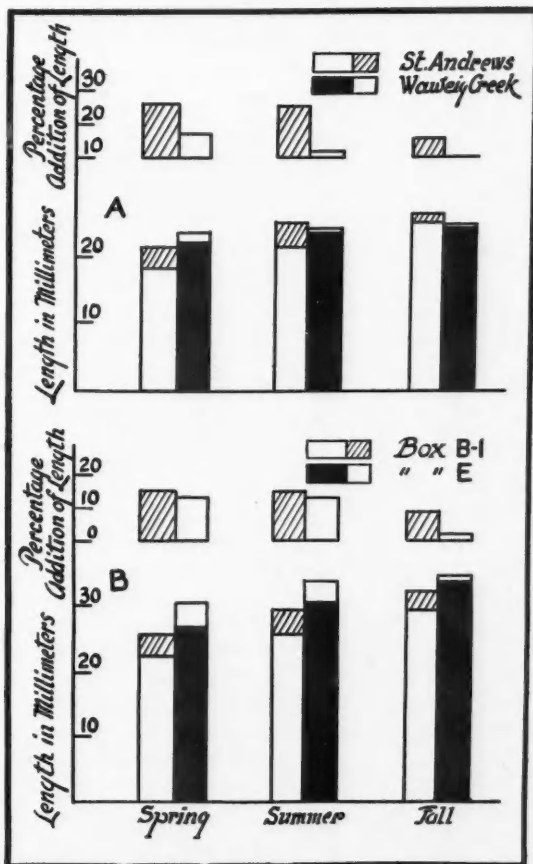


FIG. 7. Comparison of the amount of growth of *Mya arenaria* under:— A. a condition of low salinity (Waukeg C.) with that under a condition of normal salinity (St. Andrews); B. a condition of high sulphide content (Box E) with that under normal conditions (Box B-I).

A better controlled experiment on the salinity factor was carried out by the writer in the fall of 1930 (September 1 to October 15) at the St. Andrews Laboratory (16). Two lots of small clams ($n=15$; initial mean lengths, 15.6 mm. and 13.6 mm. respectively) were planted side by side at Brandy Cove, St. Andrews, under similar growing conditions (level and soil) except that one was placed in a small seepage stream and thereby exposed to fresh water. Below the rather shallow layers of beach soil there is a solid ledge of sandstone conglomerate which precludes the possibility of the experiments being affected by fresh subsurface waters. At the end of the experiment, which lasted for 43 days, Box J in the seepage stream contained eleven

TABLE XIX

CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF *Mya arenaria* USED IN EXPERIMENTS OF 1930. PERIOD; SEPT. 1,—OCT. 15. $N = 15$.

Box I			Box J		
Initial mean length	Standard deviation	C.V.	Initial mean length	Standard deviation	C.V.
15.6 \pm 0.2	0.65 \pm 0.11	4.2 \pm 0.8	13.6 \pm 0.1	0.45 \pm 0.08	3.3 \pm 0.6
Final mean length					
17.4 \pm 0.2	0.80 \pm 0.15	4.6 \pm 0.9	No growth		

specimens which were still alive but which showed no evidence of growth (Table XIX). In the control, Box I, only two clams were dead and the living ones exhibited an average growth in length of 1.8 mm. (11.5%), which is normal growth for this high level. (Compare (22) and (23)).

(3) *Sulphide content.* On the beaches of the Bay of Fundy region it is not unusual to find a black sub-surface layer rich in sulphides and decomposing materials. The influence of this particular condition on the clam fauna is problematical. To ascertain its effect on the growth of *Mya arenaria*, a group of clams (Box E) was planted at a place on the shore near the opening of the St. Andrews sewer to Passamaquoddy Bay (level, 7 feet). The lower layers of soil here were characterized by over 50 volumes per cent of sulphide, a very foul odor, and decaying matter. In spite of these conditions a few clams were found living at a distance of about 0.5 metres from the box.

The amount of growth obtained for the entire period was nearly 10 mm. (44.3%) (Table XII). The result compares quite favorably with the increment obtained elsewhere under favorable conditions (10).

The seasonal growth rate under these conditions is compared with that at a similar level (Box B 1) at Clam Cove (Fig. 7, B). Their percentage additions during the spring and summer seasons are quite similar (Table XII). In the fall, however, a difference in the absolute increment of length of about 2 mm. (7.6%) occurs. This represents the largest fall growth obtained in our experiments during the

TABLE XX
AMOUNT OF GROWTH OBTAINED IN THE
SULPHIDE EXPERIMENT
(Box E—level, 7 feet)

Season	Average length at end of each season, mm.	Seasonal absolute increment of growth, mm.	Seasonal percentage addition of length
Year 1931			
Spring	25.7	3.5	15.7
Summer	29.4	3.7	14.5
Fall	32.0	2.6	8.8
Year 1932			
Spring	35.2	3.2	9.9
Summer	37.9	2.7	7.7
Total		15.7	70.7

same period (nearly 9%). The experiment (Box E) was continued throughout the spring and summer periods of 1932. As during 1931, the spring growth slightly exceeded that taking place during the summer period, the absolute increments being 3.2 mm. and 2.7 mm. respectively (Table XX).

From these results, it is apparent that *Mya arenaria* is resistant to a wide range of environmental conditions. In their interpretation, it must be recognized that the so-called limiting factors are not to be considered as isolated environmental units. On the contrary, they are regarded only as the most significant variables and as such must materially modify the incidence of one or more other coexisting factors.

TABLE XXI

SEASONS, THEIR DURATION AND TEMPERATURE,* DURING EXPERIMENTS OF 1931 AND 1932 IN ST. ANDREWS REGION

Season	Duration of season	Number of days	Mean daily temperature, 1931		Mean daily temperature, 1932	
			Water, °C.	Air, °C.	Water, °C.	Air, °C.
Spring						
Total period	March 1 to June 19	111	4.97	6.95	3.57	4.04
Growth period	May 1 to June 19	50	7.62	8.04	7.19	10.59
Summer	June 20 to August 17	59	12.11	17.97	11.45	16.81
Fall	August 18 to October 14	57	12.31	13.64	12.83	13.99
Late fall	October 15 to December 3	50	9.06	6.30		

* Seasonal mean water temperatures represent the mean of daily mean temperatures based on the average of two readings taken about 8 a.m. and 5 p.m. Mean air temperatures represent the mean of daily mean temperatures obtained by calculating the mean of the maximum and minimum daily temperatures.

V. Discussion

The results are discussed under two main headings, namely, growth in nature, and experimental field growth.

1. GROWTH IN NATURE

In view of the absence of a satisfactory method for the determination of the age of *Mya arenaria*, an attempt has been made to evaluate the age and rate of growth in nature through a study of periodic (annual) rings on the valves. Difficulty has been experienced in recognizing the first (sometimes the second also) rings on large specimens due to a wearing away of the shell surface in the umbo region. The value for the first ring, namely, 3.9 mm., at Clam Cove (4.8 mm. at the Sissiboo River, N.S.) was obtained by measuring the single ring on specimens spawned in 1931 (Table V). Obviously, this value is not directly comparable with figures presented for succeeding rings since it represents the amount of growth during a single season. The growth during the first season may be expected to exhibit marked variation in the different parts of the Bay on account of the susceptibility of the organ-

ism at this early period of development to local variations of the physical environment which at a later stage would be less liable to prove effective. The remaining values are based on measurements of specimens of different ages, hence the increments obtained represent the mean amount of growth under average climatic conditions for the region under consideration. It is not usually possible to apply the "annual ring" method for estimating growth increments after the eighth year, because of the relatively small additions and, in many instances, the indistinct nature of the rings.

"Annual ring" measurements have been made of five collections from four widely separated beaches on the Bay of Fundy. Results indicate fairly similar values for the rings of the different collections with the exception of ring Number 2 of the Clam Cove sample. The value obtained was 15.6 mm. as compared with approximately 20.0 mm. for the other regions (Tables II, V and VI). On the basis of our present knowledge we are unable to explain this difference. (Cf. (7) and (11)).

Recognition of the existence of annual rings in *Mya arenaria* has permitted an estimation of the seasonal growth during the spring period of 1932 in the case of clams ranging from 8 to about 50 millimetres in length (Table XV). Although the number of specimens in some instances is small, they are quite reliable on account of the small variation within each group. As previously pointed out in detail, these results corroborate those obtained from planting experiments and clearly demonstrate the reduction in growth rate of approximately 50% with increase in level from 7 ft. to 18 ft. above chart datum. The trend of the decline in percentage increment with increase in size is clearly shown.

The results of annual-ring measurements of collections at levels 7 ft. and 18 ft. at Clam Cove illustrate the following points. Firstly, the growth rate at a low level on the beach is approximately double that occurring at the upper levels, thus corroborating the results of seasonal (spring, 1932) growth under both natural and experimental conditions (Tables XV and XVI). Second, the correspondence between the annual growth during 1931 as shown by annual-ring measurements and that determined by planting experiments is significant and, furthermore, strengthens the validity of seasonal increments obtained experimentally in this investigation. Thus, although the initial lengths are not sufficiently similar to permit a direct comparison of the annual increments obtained in 1931 (Table XII) with the results of annual-ring measurements (Table V), nevertheless a fairly good correspondence is evident.

Growth of small clams (initial length, 18.3 mm.) at the higher level during 1931 was 5.5 mm.; equivalent to a percentage addition of 30.0. Referring to Table V, it is seen that for clams possessing a similar initial length, the growth in nature falls about midway between 42.3% and 25.1%, probably quite close to 30%. For the seven-foot level, the absolute increment of growth in Box B was 12.0 mm., whereas the corresponding increment of growth in nature falls around 11.5 mm. (Table V). From Table XII, it is

seen that the percentage increment for large clams (initial length, 26.4 mm.) at the upper level during 1931 was 20. In nature (Table V) for a similar initial length the percentage addition lies between 25.1 and 15.1. Specimens of similar initial length, grown at a low level, show a somewhat less percentage increment than that indicated by annual-ring measurements. However, this difference may be expected since growth results obtained in experimental boxes are likely to be somewhat below normal growth in nature owing to disturbance for purposes of measurement. Third, the amount of spring growth during 1932 as indicated by annual-ring measurements is essentially in agreement with the results obtained from planting experiments (Table XVI).

The rate of growth in this species shows marked variation depending on age. The growth rate during early life decreases very rapidly with a subsequent less rapid decline (cf. Fig. 3). A similar trend of growth has been obtained by various authors working with molluscs and several species of fish (cf. 29, p. 61).

2. EXPERIMENTAL FIELD GROWTH

The complexity of conditions operating in nature which affects the growth rate of this species renders a definite explanation of observed phenomena difficult. However, on the basis of our present knowledge, we may at least offer a tentative interpretation of the differential growth rates evidenced by the results. An interpretation of findings based on experiments in nature, requires a recognition of the complexity of physical as well as biotic factors affecting the rate of growth and survival of the species. To isolate the several individual factors in respect to their influence on rate of growth by simulating natural conditions in the laboratory likewise presents difficulties. Hence, in consideration of these facts, care has been exercised to employ control experiments and to check results whenever possible.

It has been noted that specimens of various sizes exhibit an increase in growth with approach to the low water level (Fig. 3, C). Our results corroborate those first reported by Mead and Barnes (14) in demonstrating the fact that clams of this species submerged in water all the time grow more rapidly than those planted at any level in the intertidal zone. Stephen (28), working on *Tellina tenuis* discovered an opposite relationship between rate of growth and level, correlated with a different method of feeding. He obtained no growth below the low water mark. The variation between these two forms has been attributed to difference in food assimilated. Our results strongly suggest that organic detritus does not constitute a very important part of the food of *Mya arenaria* in this region; rather that the microscopic plant life (chiefly diatoms) present in the water is the dominant source of nourishment (cf. 1, 9, 24). Lotsy (13) found that a large percentage of the diatoms present in the stomach contents of *Mya arenaria* is completely digested but that the decaying organic matter shows no indication of being digested. Again, it has been demonstrated that the clam in this region is quite sensitive to variation in exposure and thus, we believe, to changing abundance of food.

Hunt (8) states "such a seasonal fluctuation of their diet may be an immediate factor concerned in the seasonal variation in growth of marine animals . . . it seems likely that a rapid growth rate is correlated with a diet rich in living organisms." Even if detritus were present in considerable quantities in the stomach contents of *Mya arenaria* (which is contrary to our findings) we have no basis for assuming that it is digested, since Yonge (33) failed to find any evidence of the presence of cytase in this species.

The results obtained from a study of annual rings and from field experiments indicate the effect of size on relative growth rate in *Mya arenaria*.

It has been observed that variations occur in seasonal growth on different beaches as well as various levels of the same beach. Furthermore, results point to a variation in duration and amount of growth during the fall and winter of 1930 as compared with the corresponding seasons of 1931. It may be in order to consider first the more general aspects of the findings pertaining to distribution of growth throughout the year 1931, followed by a more detailed discussion of the variation between the several levels on the beach as well as a comparison of the growth increments during corresponding seasons of 1931 and 1932.

In 1931 the seasons of greatest growth were spring and summer. With hardly an exception (note Box E) the length increment after about August 20 (fall season) is relatively small. This fact is clearly shown in Fig. 4, A, in which the seasonal growth of small and large clams is compared. During the late fall period, no significant growth occurred. These data are seemingly contrary to expectations in view of the growth occurring during the spring period when the mean water temperature was comparatively low, namely, 7.6° C. (Table XXI). The spring period, extending from about March 1 to June 19, although much longer than any one of the other periods, probably represents similar time for growth, 50 days. However, on the basis of previous work it appears unlikely that much growth takes place before March 1. Thus, the mean water temperature during April, 3.7° C., was relatively low for growth; in May it was 6.6° C. and in June, 9.6° C. (Table X). Belding (1) states that *Mya arenaria* continues to grow until a temperature of 42° to 40° F. (5.6° to 4.4° C.) is reached. Hence, in view of the low mean temperature for April (3.7° C.), we believe that growth did not start until May and, therefore, results for the spring season represent growth occurring after May 1 (50 days) during which time the mean daily water and air temperatures were 7.6° C. and 8.0° C. respectively (Table XXI). Comparing these mean temperatures with those for the late fall period (also 50 days) it is seen that the water temperature in spring is less while the amount of growth is in every instance far greater (no appreciable growth was obtained for the late fall season). The air temperature is 1.7° C. less during the late fall period. This fact is of little consequence here, especially in the case of clams planted at the lower levels with a very short period of exposure.

Again, comparing results for summer and fall periods of similar length, the extent of growth during summer far exceeds that taking place during the

fall period although the water temperatures are similar (12.1° and 12.3° C.). The air temperatures exhibit some variation but not sufficient to serve as factors explaining the differential growth rate.

Considering the magnitude of growth and thermal conditions during the spring and summer periods, there is a general similarity in growth rate but a wide variation in mean daily temperature of both water and air (Table XXI). These facts definitely rule out temperature as the direct determining factor.

The data indicate that some factor besides temperature must be operating to cause these seasonal variations in rate of growth. The question arises as to why the growth rate diminished in September and October and ceased almost entirely during the late fall period. This question can not be definitely answered, however certain lines of evidence are available which support the contention that food supply is largely responsible for the differential rate.

First, mention may be made of the investigations of Kellogg (9) and Belding (1), both of whom consider rate of growth in *Mya arenaria* to be directly correlated with the quantity of food. Wilton and Wilton (32) present evidence to suggest that clams are quite sensitive to variability in their food supply. Second, the seasonal fluctuations in diatom abundance evidence a significant correspondence with growth variability. The intention here is to point out some of the more cogent facts relative to diatom abundance that throw light on the problem in question.

It is stated in a previous paragraph that during the fall period of 1931 the amount of growth was significantly less than that of the corresponding period for 1930. Furthermore, growth continued until about December 1 during 1930, whereas practically none occurred after the middle of October, 1931 (late fall period). For an explanation, attention is turned to temperature and food as the most likely sources.

Regarding temperature, it has been found that the mean daily water temperature during September, 1930, was 13.4° C. as compared with 12.2° C. during September, 1931 (Table X, Fig. 8). As is shown in Fig. 8, the temperatures during the remaining fall months are similar for both years. It seems apparent, therefore, that temperature is not the explanation for the small amount of fall growth during 1931. Likewise, Lea (11) working on seasonal growth rates in herrings clearly points out that these rates are not correlative with the temperature. He states "comparing two periods with approximately the same temperature, e.g., June and October, it is seen . . . when the temperature in October becomes the same as in June when the rate of growth reached its maximum, the rate of growth is well on its way to the minimum".

In considering the food factor, it may be pointed out in the beginning that sufficient data are not available pertaining to the seasonal or annual abundance of diatoms in the immediate vicinity of the clam beaches under consideration, to warrant definite conclusions. We are particularly fortunate in having access to qualitative and quantitative records collected over a period of several years, by Doctor Viola M. Davidson.

The data which have been obtained from Dr. Davidson were collected at Station 6, located near the middle of the St. Croix River about 800 metres from the clam beaches near the Biological Station, St. Andrews. From the viewpoint of their effect on the abundance of diatoms, conditions operating at this station may be considered similar to those in the waters over the clam beds, owing to the uniformity of nutrient salts at the various depths produced

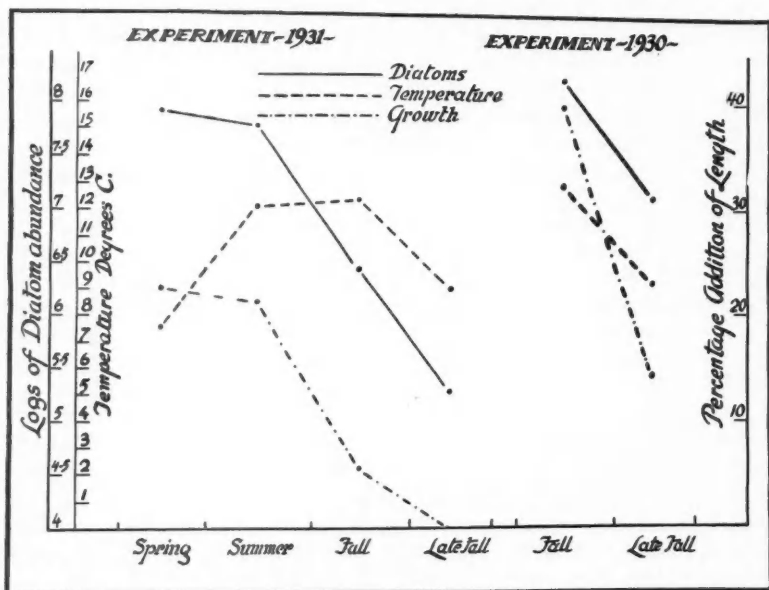


FIG. 8. Fluctuations in diatom abundance, temperature, and rate of growth of *Mya arenaria* during periods of experiments of 1930 and 1931. The diatom curve is logarithmic, being based on the geometric means of weekly records obtained at Station 6, St. Croix River, N.B.

by the intensive mixing characteristic of this region. Thus, with an absence of pronounced stratification such as is encountered in the Gulf of Maine waters, we can reasonably expect the demersal and neritic forms to be affected in about the same way as the deeper water oceanic species*. From these data there has been prepared a logarithmic curve of diatom abundance during the periods of our experiments of 1930 and 1931 (Fig. 8). According to Dr. Davidson, the curve of diatom abundance for 1930 is exceptional, the fall figures exceeding those of any year since 1924. The 1931 curve depicts a somewhat different trend throughout the seasons. It is seen that there was a pronounced decrease during the fall and late fall periods of 1931 which is directly correlated with a pronounced decrease in growth rate. The small amount of growth that is apparent from our results for the period from the

*Personal communication from Dr. Braarud.

middle of October, 1930, to about March 1, 1931 (2.8 mm.) is apparently due to the low temperatures following December 1, since diatoms were abundant during February (4).

We have considered evidence pertaining to the importance of food in accounting for the difference in rate of growth during the fall periods of 1930 and 1931. This has suggested its possible importance in affecting the seasonal growth rate during 1931.

Comparing the growth curve with the curve of diatom abundance for 1931 (Fig. 8), similar trends are observed. It is noted that the peak of diatom abundance corresponds with the rapid growth of the spring season (the principal growth during this season is considered to occur in May. See above). The average diatom abundance during the summer season, in which growth is quite similar to that of the spring period, is fairly high, and it is very low in the late fall period when growth is approximately nil. On the basis of the diatom abundance, a somewhat higher rate of growth during the fall period might be expected. Growth data for the spring and summer periods of 1932 also show a rapid rate which likewise corresponds with a similar trend of diatom abundance.

An attempt to confirm the importance of diatoms as food was undertaken during the summer of 1932. As a result of qualitative comparisons (quantitative data only relative) between the planktons of the surface and bottom layers of water and those of the stomach contents of this species, it has been demonstrated that bottom forms which are also frequently found in the plankton (*Paralia sulcata*, *Pleurosigma* sp. and *Navicula* sp.) and neritic species are most abundant in the stomach contents.

Of the zooplanktons present, the tintinnids occur in greatest abundance, the common species being *Tintinnus subulatus* and species of *Tintinnopsis*.

To recapitulate, our data indicate that growth, during the months from December to April, inclusive, is practically negligible owing to the mean water temperature (air temperature also) being considerably below 5.0° C. and that seasonal growth during the remaining months of the year is not correlated directly with these conditions, but rather with the food supply.

The average results obtained for the beach at Clam Cove during 1931 (Table XII and Fig. 4, B) show that the percentage additions of length during spring and summer were approximately the same in the case of small and large specimens and that there was, in both sizes, a very evident decrease during the fall of 1931. A corroboration of these indications was obtained in field experiments during the spring period of 1932 (Table XIV). The percentage increment of growth, 9.3 mm., was only slightly higher than that for the summer season (8.6%) when the mean daily temperatures were appreciably higher (Table XXI). According to Dr. Davidson* the abundance of diatoms in the spring period was high and followed a trend quite similar to that of 1931 (Fig. 8) and hence corresponded with the amount of growth obtained. Similar results have been obtained in comparable experiments

*Private communication.

during the spring seasons of 1931 and 1932. During the summer period of 1932, a somewhat lower rate has been obtained than during the corresponding period of 1931 (Table XIV). The amounts of spring growth during 1932 as evidenced by the results of planting experiments and annual-ring measurements have been directly compared in Table XVI and a very good correspondence obtained. By means of ring measurements it has been possible to present in Table XV a picture of the spring growth in 1932 over a fairly wide range of size in the case of clams growing at both high and low levels of the beach at Clam Cove, N.B.

The effect of exposure in limiting seasonal growth on the beach near St. Andrews is shown in Fig. 5, C and D. Annual-ring measurements corroborate the results of planting experiments in pointing to an approximate 50% reduction in annual growth rate accompanying an increase in level from 7 to 18 ft. above chart datum (Tables V and XV). It should be remembered that exposure not only restricts feeding time but also aeration, and therefore metabolic rate. However, this might not prove significant in limiting the growth processes, providing an adequate food supply was available, since Collip (2) was able to demonstrate that clams possess a source of oxygen, which he considers may be a compound in the tissue, of the nature of an organic peroxide, that is available for metabolic needs during long periods of oxygen insufficiency in the enveloping medium. In the case of *Tellina tenuis* which feeds on detritus, Stephen (26) has obtained greatest growths at levels exposed for long periods. Thus, a decrease in aeration does not seem to be detrimental. Our results do not show that detritus is an important source of food for *Mya arenaria* (cf. 33). On the basis of these observations, it seems safe to conclude that the restricting effect of exposure is caused not by decrease in aeration, but rather by an accompanying reduction in the available food supply.

(b) Unfavorable Conditions

Important unfavorable conditions in nature which have received attention are soil, salinity, and sulphides.

Soil. Two types of soil which have been found to exert a limiting influence on the growth of *Mya arenaria* are (i) soil consisting of a large percentage of mud mixed with sand and covered with a layer of fine silt and (ii) fine shifting sand.

In the case of the first type, the retardation of growth is attributed to the mechanical action of the fine silt which interferes with the normal feeding processes (cf. 33). Results obtained in Boxes G and H planted at Cranberry Point and Harbor de Lutre (difference in percentage addition of length, 4.5) demonstrate the retarding influence. Furthermore, at Clam Cove, specimens in Box C, although planted at a slightly lower level than those of Box B, nevertheless grew at a slower rate throughout the principal growing seasons, the difference in total percentage addition of length for the entire period being 8.8. The most pronounced difference in the absolute increment of

growth (2.3 mm.) equivalent to a percentage addition of length of 9.2, occurred during the summer season. Apart from the limiting effect on growth, there has been noted a scarcity of clams in this type of beach (soft mud), indicating an unfavorable condition for survival of the set.

On the beaches consisting of fine shifting sand, clams are not only absent but fail to survive when planted. In the experiment carried on at Joe's Point during a period of 65 days only 27% of the clams survived. This is due to the siphons becoming filled with sand which the clam is unable to remove.

Salinity. That *Mya arenaria* is capable of resisting a wide range of salinity is very well known (1). However, results show that clams growing in nature may evidence a distinct retardation when the range is extreme (2 o/oo to 30 o/oo) (cf. 22 and 23).

Sulphide content. Results obtained in the case of clams planted near the mouth of a sewer where the sulphide content of the black soil was over 50 volumes per cent, show, contrary to expectations, a growth quite comparable to that occurring under favorable conditions in Box B 1 at Clam Cove (Table XII and Fig. 7, B). The data obtained indicate the high resistance of *Mya arenaria* to high sulphide concentration which is found on quite a number of beaches. In view of the complexity of the chemical changes operating in the black layers, it is difficult to explain the action of sulphides on the growth processes (cf. 18).

VI. Summary and Conclusions

The results of this study may be summarized as follows:

1. GROWTH IN NATURE

1. *Mya arenaria* possesses "annual rings" on the valves which serve as a criterion for the determination of age and rate of growth. Frequently difficulty is experienced in recognizing the first ring which suggests the amount of growth during the first year, owing largely to a wearing away of the outer shell layer in the umbo region.

2. Study of the annual rings shows that growth activity per unit of length exhibits a marked decline with increase in age from a very early period. The percentage additions of length obtained by averaging results from four Bay of Fundy samples are:—during the second year, 346.3; the third year, 59.9; the fourth year, 33.3; the fifth year, 19.6; the sixth year, 14.3; and the seventh year, 10.7.

2. EXPERIMENTAL FIELD GROWTH

3. In the experiments of 1931, clams of two sizes were selected; "small" (17 to 20 mm. in length) about two years old, and "large" (25 to 28 mm. long) three years old. For an understanding of the role played by environmental factors which influence growth, two series of experiments have been carried out under: (a) Normal conditions; (b) Limiting conditions.

(a) *Normal Conditions*

4. Under favorable conditions, the factors considered are submergence, food and temperature. The beaches studied are located at Clam Cove, Deer Island, and near St. Andrews. Boxes were planted at three levels: high, 18 to 19 ft. above chart datum; medium, 13 ft.; and low, 5 to 7 ft., on experimental beaches. In general, small clams (18.4 mm. in length) about two years old exhibit a faster rate of growth than large individuals (26.4 mm. long) three years old, throughout each season; the maximal annual absolute increment of growth for small clams was 12.8 mm. (percentage addition of length, 66.2) and 8.9 mm. (percentage addition of length, 33.5) in the case of large clams.

5. A comparison of the growth rate during the third and fourth years, obtained in growth experiments during 1931, with that calculated from the estimated annual rings of specimens growing at comparable levels, has yielded similar results (page 117), namely, an absolute increment of length during the third year of about 13.0 mm. and during the fourth year of approximately 11.0 mm.

6. The rate of growth of small and large clams varies inversely with the beach level. Regarding small clams planted at Clam Cove, the annual growth at a level of eighteen feet above chart datum was less than one-half that occurring at a level of seven feet. In the case of large clams at corresponding levels, the reduction was about one-third.

7. The principal growth took place from the beginning of May until September (spring and summer seasons) when approximately 92% of the yearly growth for small and large specimens occurred. It may be mentioned that the amount of growth during the spring season was similar to that taking place during the summer period. This occurred in the case of both small and large clams, *e.g.*, the percentage additions of length during each of the aforementioned seasons for small clams in Box C was about 20, for large clams in Box B 1 about 13.

8. The amount of growth during the spring seasons of 1931 and 1932 was quite similar, whereas in the case of the summer periods, a slight decline was shown during 1932.

9. Very little growth occurred during the fall of 1931 in comparison with the corresponding season of 1930 when growth continued until about December 1 (Fig. 8).

10. The following explanations are offered for the variations in seasonal growth which occurred during 1930 and 1931, as well as the difference in fall growth during both years:

(a) Practical absence of growth during the winter season (about December to April, 1931) is attributed chiefly to low temperature.

(b) Variation in growth throughout the entire year at different levels is directly correlated with the time of submergence. Thus, in 1930, clams sub-

merged all the time showed greater growth than those planted at any level on the intertidal zone.

(c) Variation in the amount of growth during the different seasons of 1931 and that in the fall seasons of 1930 and 1931 is attributed chiefly to fluctuations in the amount of food (diatoms) present. According to the investigation of Doctor V. M. Davidson, the quantity of diatoms in plankton of the Passamaquoddy region in the fall of 1930 was exceptionally high as compared with that of 1931 (Fig. 8). Also, the abundance of diatoms during the spring and summer months of 1931 was much greater than during the fall of this year, thus indicating a close correspondence with the known variations in rate of growth. Available data for the spring and summer periods of 1932 indicate a similar trend.

11. Of the plankton constituents in the stomach contents of *Mya arenaria*, diatoms have been found to be most abundant, and zooplanktons, chiefly tintinnids, play a minor food role. The diatoms taken in by *Mya* are, in the main, bottom forms which also occur frequently in the plankton (*Paralia sulcata*, *Pleurosigma* sp. and *Navicula*) and neritic species.

12. Experiments designed to analyze the resistance of *Mya arenaria* to unfavorable conditions of soil, salinity, and sulphide content were set up on beaches especially suitable for this purpose (Table XVIII).

13. Two types of soil limit the growth rate of this species (a) fine shifting sand, (b) soil consisting of a high percentage of mud with a surface layer of fine silt.

14. Water possessing a very low salt content is unfavorable for growth. However, the resistance of *Mya* to this factor is so great that the usual range of salinity encountered on commercial beaches of the Bay of Fundy region cannot be considered an important factor in determining differential growth rate.

15. The growth rate of clams is not significantly retarded by the sulphide content present in the soil of clam beaches of the Bay of Fundy.

Acknowledgments

The writer wishes to express his appreciation and thanks to Doctor A. G. Huntsman, Professor of Marine Biology at the University of Toronto, for advice and co-operation. Acknowledgment is also made to Doctor V. D. Vladikov for his helpful suggestions and interest in the problem; to Doctor Viola M. Davidson who has been most liberal in permitting the use of her original data; to Doctor Tryge Braarud for identifying diatoms; to Doctor T. B. Clark for checking certain statistical methods; to Mr. Oscar Shretter for the preparation of drawings; and to several investigators at the Atlantic Biological Station, particularly the late Professor A. Brooker Klugh and Professor H. B. Hachey, for valuable advice.

The writer is indebted to the Biological Board of Canada for laboratory facilities during the experimental work; to the National Research Council of Canada for financial aid; and to Professor E. A. Andrews through whose kindness the author has had facilities for the continuation of this study in the Zoological Laboratories of the Johns Hopkins University.

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